Highly potent and selective acylguanidine-type histamine H_2 receptor agonists: synthesis and structure-activity relationships of mono- and bivalent ligands

Dissertation

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Contents

Abbreviations

abs	absolute
AC	adenylylcyclase
Am	2-aminothiazole
aq.	aqueous
Ar	aromatic
ATP	adenosine triphosphate
Boc	*tert*-butoxycarbonyl
BRET	bioluminescence resonance energy transfer
BSA	bovine serum albumin
cAMP	cyclic 3`,5`-adenosine monophosphate
cat.	catalytical amounts
Cbz	benzyloxycarbonyl
CDI	*N*,*N*`-carbonyldiimidazole
cHex	cyclohexyl
CI	chemical ionization
CNS	central nervous system
conc	concentrated
COSY	correlated spectroscopy
CRE	cAMP response element
CREB	cAMP response element binding protein
d	day(s) or doublet
DAG	diacylglycerol
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimetheylsulfoxide
DMSO-d_6	per-deuterated DMSO
e2	second extracellular loop of a G-protein coupled receptor
EC_{50}	molar concentration of the agonist causing 50 % of the

	maximal response
EDAC	*N*-(3-dimethylaminopropyl)-*N*`-ethylcarbodiimide
EI	electron impair ionization
E_{max}	maximal response relative to histamine (1.00)
eq	equivalents
ES	electrospray ionization
EtOAc	ethylacetate
Et_2O	diethyl ether
FRET	fluorescence resonance energy transfer
GDP	guanosine diphosphate
GEF	guanine nucleotide exchange factor
GF / C	a glass fibre filter grade (1.2 µm)
GPCR	G-protein coupled receptor
gp	guinea pig
gpH_2R	gp histamine H_2 receptor
gpH_2R-$Gs\alpha_S$	fusion protein of the gpH_2R and the short splice variant of Gsα
gpH_2R-hE2- $Gs\alpha_S$	fusion protein of the gpH_2R bearing Asp-169→Gly167, Asp-169→His-169, Ile-171→Thr-171 and Val-172→Ser-172 mutations and the short splice variant of Gsα
$Gs\alpha_S$	short splice variant of Gsα
GTP	guanosine triphosphate
h	hour(s) or human
HR	histamine receptor
H_2R	histamine H_2R
hH_1R	human histamine H_1 receptor
hH_2R	human histamine H_2 receptor
hH_2R-$Gs\alpha_S$	fusion protein of the hH_2R and the short splice variant of Gsα
hH_2R-C17Y-$Gs\alpha_S$	fusion protein of the human H_2R bearing a Cys-17→Tyr-17 mutation and the short splice variant of Gsα
hH_2R-C17Y-A271D-$Gs\alpha_S$	fusion protein of the human H_2R bearing a Cys-

	17→Tyr-17 and Ala-271→Asp-271 mutation and the short splice variant of Gsα
hH_2R-gpE2-$Gs\alpha_S$	fusion protein of the human H_2R bearing Gly-169→Asp167, His-169→Asp-169, Thr-171→Ile-171 and Ser-172→Val-172 mutations and the short splice variant of Gsα
hH_3R	human histamine H_3 receptor
hH_4R	human histamine H_4 receptor
hH_4R-RGS19	fusion protein of the hH_4R and RGS19
HPLC	high performance (pressure) liquid chromatography
HR-MS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
HT-29	human colon carcinoma cells
IC_{50}	antagonist (inverse agonist) concentration suppressing 50 % of an agonist induced effect
Im	imidazole
IP_3	inositol-1,4,5-trisphosphate
J	coupling constant
k`	capacity factor
K_B	dissociation constant (functional assay)
LSI	liquid secondary ion
m	multiplet
MAPK	mitogen-activated protein kinase
min	minute(s)
mp	melting point
MS	mass spectroscopy
N	nitrogen
N^G	guanidino-nitrogen
NHS	N-hydroxysuccinimide
NMR	nuclear magnetic resonance
PE	petroleum ether
Ph	Phenyl
P_i	inorganic phosphate
PIP_2	phosphatidylinositol-4,5-bisphosphate

PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase
ppm	part per million
py-1	2,6-dimethyl-4-[(E)-2-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-ij]quinolin-9-yl)-vinyl]pyranylium tetrafluoro borate
Pip	piperidine
Phth	phthalimide
pEC_{50}	negative decadic logarithm of EC_{50}
pK_B	negative decadic logarithm of K_B
q	quartet
quat	quaternary
R	receptor
R	inactive state of a GPCR
R^*	active state of a GPCR
RGS	regulator of G-protein signaling
RP	reverse phase
rt	room temperature
rpm	rounds per minute
s	singlet
SEM	standard error of the mean
Sf9	*Spodoptera frugiperda* insect cell line
t	triplet
t_0	dead time
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thiaz	thiazolyl
TLC	thin layer chromatography
TM	transmembrane domain of a GPCR
TM1-TM7	numbering of transmembrane domains of a GPCR
t_R	retention time
Triaz	1*H*-1,2,4-triazolyl

Chapter 1

Introduction

1.1 G-protein coupled receptors

1.1.1 GPCRs as drug targets and their classification

G-protein coupled receptors (GPCRs) constitute the largest group of integral membrane proteins, accounting for approximately 2–3 % of the human genome.[1] GPCRs transduce signals through a wide range of effectors influencing a multitude of important physiological functions. The involvement in several diseases including pain, asthma, inflammation, obesity, cancer, as well as cardiovascular, metabolic, gastrointestinal and CNS diseases[2] makes them one of the most important classes of drug targets. It is estimated that more than 30 % of the currently marketed therapeutic agents modulate GPCR activity.[3-4] Half of approximately 800 identified GPCRs are chemosensory receptors (csGPCRs) and respond to external signals such as pheromones, odors, tastes or photons,[5-6] The remaining receptors are addressed by endogenous ligands, for instance, peptides, lipids, neurotransmitter and nucleotides (endoGPCRs).[4] For 140 of these endoGPCRs the endogenous ligands are not known to date, referred to as “orphan receptors”.[7-10] Based on structural differences, mammalian GPCRs can be divided in five main families termed rhodopsin, secretin, adhesion, glutamate and frizzled/taste2.[4] The rhodopsin-like family, also referred to as class A of GPCRs, is by far the largest and best studied subgroup containing receptors for odorants, small molecules such as biogenic amines, peptides and glycoprotein hormones (≈ 700 GPCRs, including csGPCRs and endoGPCRs). The binding sites of small endogenous ligands are located within the seven transmembrane (TM) domains, whereas binding of more space filling ligands, for example peptides and glycoproteins, occurs at the amino terminus (N-terminus), extracellular loops and amino acids located at the top of the TM helices.[5] The secretin-

like receptor family (class B) contains 15 members including GPCRs for the peptides secretin, calcitonin and parathyroid hormone. A large N-terminus, which is involved in ligand binding, is characteristic of these receptors. The third main class of GPCRs is the glutamate receptor family (class C), implying the metabotropic glutamate receptor, the γ-aminobutyric acid type B ($GABA_B$) receptor and Ca^{2+}-sensing receptors. Herein, the ligands bind in the very large N-terminal region, which has a characteristic structure known as the "Venus flytrap" module.[3] Finally, the members of the adhesion GPCRs are thought to participate in cell adhesion, the frizzled and smoothened receptors play a role in cell development and proliferation and the members of the taste2 receptor family are crucial for the detection of the bitter taste of substances.[1,3-5] All members of the GPCR superfamily share a common architecture. This structural feature is the presence of seven hydrophobic membrane-spanning α-helical segments, the transmembrane domains, which are connected by three intracellular and three extracellular loops. The N-terminus is on the extracellular side whereas the carboxy terminus (C-terminus) is intracellular. Besides the structural requirement of seven TM domains, the receptor has to interact with a heterotrimeric G-protein, located on the intracellular side, to be classified as GPCR. But, given that G-protein independent signaling pathways are demonstrated for some of these receptors (see 1.1.3.2),[1,11] the term seven transmembrane receptors (7TMRs) would be more appropriate.

The determination of the crystal structure of bovine rhodopsin by Palczewski in 2000[12] provided insight into the three dimensional architecture of a mammalian class A GPCR and offered new opportunities for GPCR research. This structure served as template for homology models to study GPCR conformations and ligand-receptor interaction on the molecular level. Recently, further crystal structures have been resolved including the human β_2-adrenergic receptor,[13-18] the turkey β_1-adrenergic receptor,[19] the human adenosine A_{2A} receptor,[20] the human dopamine D_3 receptor,[21] the human histamine H_1 receptor[22] and opsin, the first receptor crystallized in its active state.[23-24] The ionic-lock (salt bridge between Arg-131 (TM3) and Glu-268 (TM6)) which is suggested to stabilize the inactive conformation of rhodopsin and the D_3R,[12,21] was broken in all other GPCRs.[13,15,19-20,22,25] In addition, observations like the presence of an α-helix in the second extracellular (e2) loop in the adrenergic receptors,[13,15] constrain the crystal structure of rhodopsin as ideal representative for other GPCRs.[26] Thus, the very recently

elucidated crystal structures will contribute to improved homology models and consequently facilitate the target-based drug design for many GPCRs.

1.1.2 GPCR activation and ligand classification

Several models have been proposed for the molecular mechanism involved in the activation of GPCRs upon interaction with appropriate ligands. Amongst them, the extended ternary complex model[27-29] is considered most suitable for explaining the pharmacodynamic activities of the majority of interacting ligands. According to this model, GPCRs exist in an inactive conformation (R) and an active conformation (R^*) that efficiently couples to a defined G-protein (G), leading to the functional species (R^*G). In a given environment, equilibrium spontaneously establishes between the usually predominant inactive and the active conformation. The inactive form is allowed to isomerize to an active form independently from agonist binding. This spontaneous activation of the receptor in the absence of agonists is referred to as constitutive activity.[30]

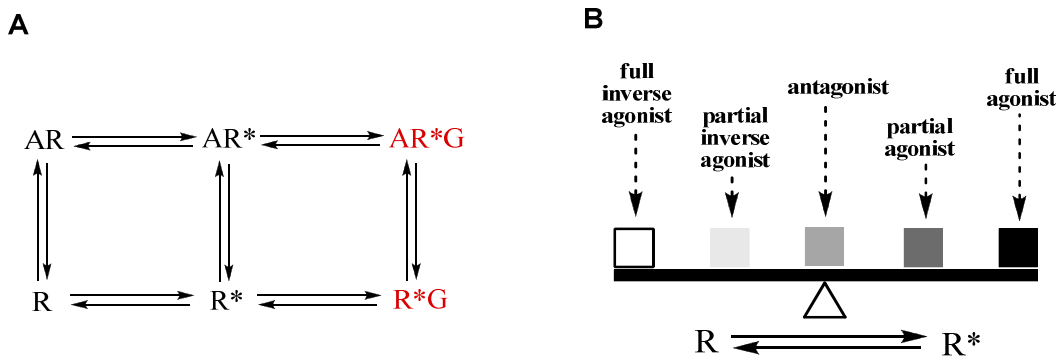


Figure 1.1. Two-state model of GPCR activation. This model assumes that GPCRs isomerize from an inactive state (R) to an active state (R^*). **A)** Extended ternary complex model (R: inactive state of the receptor; R^*: active state of the receptor; G: G-protein; A: agonist). **B)** Ligand classification according to their capability of shifting the equilibrium to either side of both states. According to Seifert et al.[30]

Ligands are classified according to their capability of shifting the equilibrium to either side of both states. Agonists are ligands with higher affinity for the R^* state, stabilizing the active conformation and therefore enhancing the functional response (receptor activation). On the opposite, inverse agonists preferentially interact and stabilize the inactive conformation R of the receptor and reduce the percentage of spontaneously active receptors. Neutral antagonists bind to both conformations with the same affinity without altering the equilibrium but impairing the binding of other ligands. Partial agonists and partial inverse agonists are less effective, only partially binding and

stabilizing the active and the inactive receptor conformation, respectively.[31-32] An additional layer of complexity is added through allosteric ligands, which bind to sites that are topographically different but conformationally linked to the orthosteric site recognized by the endogenous ligand.[33] Binding to an allosteric site on a GPCR changes the receptor conformation and can modulate the binding affinity as well as the signaling efficiency of orthosteric ligands, or can perturb signaling even in the absence of orthosteric ligands.[34] Besides, the existence of ambiguous effects like "insurmountable antagonism" is discussed.[35-36] Insurmountable antagonists have the ability to depress the maximal response of orthosteric agonists and therefore do not behave as typical antagonists. This effect can be explained through the longevity of the antagonist-receptor complex, slowly interconverting receptor conformations, allosteric binding sites or receptor internalization after antagonist binding.[35]

It is apparent that the function of GPCRs is much more complex in terms of ligand binding, different conformational states, accessory protein interaction, phosphorylation, G-protein coupling, oligomerization and internalization than assumed previously.[34,37] The existence of several inactive and active receptor conformations[38] suggests that structurally different ligands stabilize distinct receptor conformations, resulting in diverse biological responses.[39] In summary, the demonstrated two-state model provides a molecular basis for classical concepts of pharmacology and helps to explain the properties of drugs acting as agonist, antagonist and inverse agonist, but the complete real situation cannot be reflected.

1.1.3 Signal transduction

1.1.3.1 G-protein mediated signal transduction

The classical model of GPCR signaling is based on the ability of these receptors to act as ligand-activated guanine nucleotide exchange factors (GEFs) for heterotrimeric G-proteins that transmit signals through the activation of intracellular effectors from the extracellular to the intracellular region.[11] These G-proteins consist of a Gα-subunit and a Gβγ-heterodimer.[40-41] The binding of the G-protein to the active conformation of the GPCR (either stabilized by an agonist or agonist-free considering constitutively active GPCRs) induces a conformational change of the G-protein and results in a rapid release of GDP from its binding site on the Gα-subunit and in the formation of the ternary

complex. The ternary complex consists of the agonist, the receptor and the nucleotide-free G-protein and is disrupted through the binding of GTP to the Gα-subunit. This exchange of GDP by GTP promotes the dissociation of the Gα-GTP-subunit and the Gβγ-complex from the receptor and from each other. Both dissociated subunits can activate or inhibit various effector proteins, like enzymes and ion channels, resulting in a variety of cellular functions. After a certain period of time, the intrinsic GTPase activity of the Gα-subunit converts GTP to GDP and phosphate. This effect is utilized in the GTPase assays applied in this work. Now, the GDP-bound Gα-subunit re-associates with Gβγ affording the next G-protein cycle.[42] The GTPase activity of Gα is accelerated by a family of proteins called the regulators of G-protein signaling (RGS).[43-45]

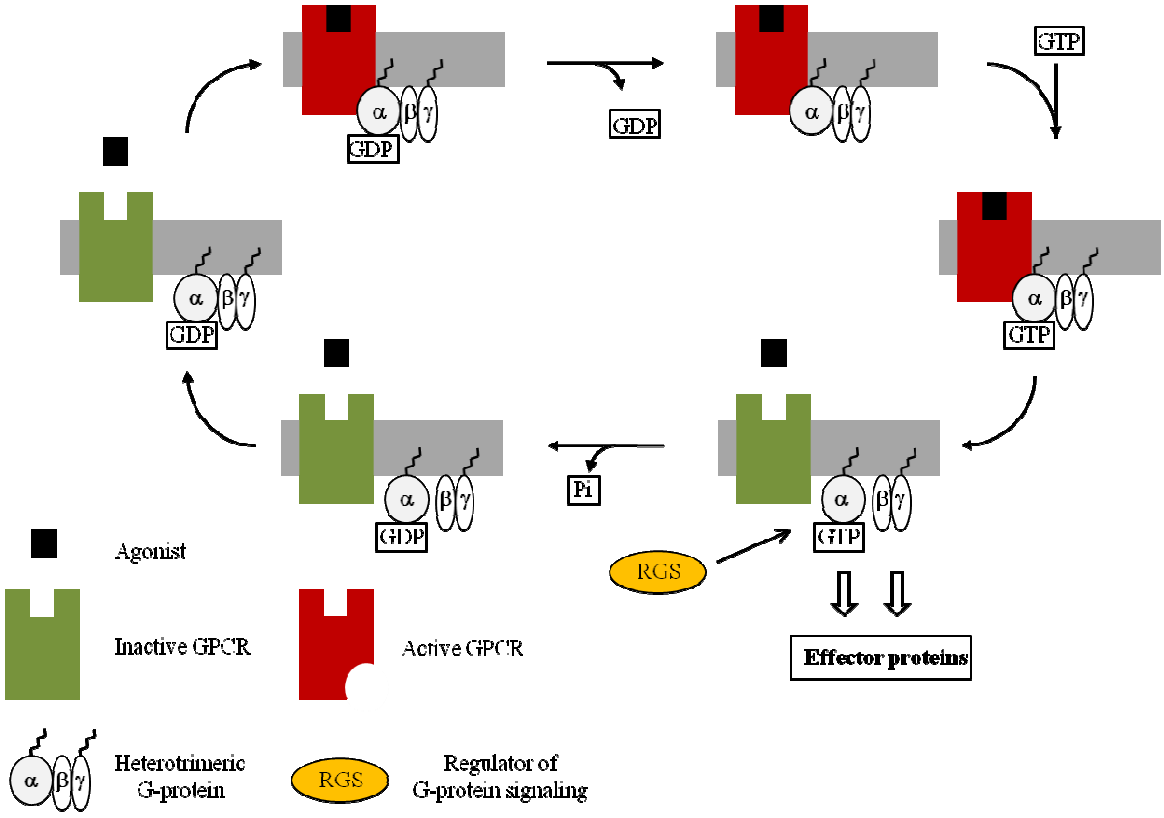


Figure 1.2. G-protein cycle.

Both, the Gα-subunit and the Gβγ-heterodimer hold lipid anchors keeping the G-proteins on the intracellular side of the membrane and in proximity to membrane proteins like GPCRs.[46] Based on their structure and signaling pathway, G-proteins are divided into four main families according to their Gα-subunit, termed G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$.[47] The $G\alpha_s$ family activates adenylyl cyclases (AC 1–9) resulting in increased cellular levels of the second messenger cAMP (3´-5´-cyclic adenosine monophosphate). In contrast to that,

the $G\alpha_i$ family shows inverse effects, inhibiting the AC activity (AC 5 and AC 6). cAMP is derived from ATP and exerts various cellular effects such as activation of the protein kinase A (PKA) or the mitogen-activated protein kinase (MAPK) pathway, both modulating gene expression.[48] For instance, PKA is a serine/threonine kinase that phosphorylates numerous substrate proteins such as the cAMP response element binding protein (CREB), affecting the gene transcription driven by the cAMP response element (CRE).[49] Inactivation of cAMP, catalyzed through phosphodiesterases, leads to termination of the signal transduction. The $G\alpha_q$ family regulates phospholipase C activity (PLC_β) resulting in hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) into the second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Elevated IP_3 levels promote the release of Ca^{2+}-ions from the intracellular endoplasmatic reticulum into the cytosol.[50] DAG and Ca^{2+}-ions stimulate the proteinkinase C (PKC), thereby modulating the function of cellular proteins by phosphorylation.[51] Finally, the $G\alpha_{12}$ family interacts with Ras homology GEFs (Rho-GEFs) that regulate cytoskeletal assembly.[5,11] In addition to the Gα-subunit, the Gβγ-heterodimer can specifically regulate certain effectors like PLC_β and ion channels.[47]

1.1.3.2 G-protein independent signaling, β-arrestin and functional selectivity

Although, the vast majority of GPCRs are able to transduce signals into cells through G-protein coupling, recent work has indicated that GPCRs participate in numerous other protein-protein interactions which generate intracellular signals in conjunction with, or even independent of, G-protein activation. Protein-protein interactions which modulate GPCR signaling include GPCR dimerization (see 1.1.4), the interaction with receptor activity-modifying proteins (RAMPs) and the binding of various scaffolding proteins to GPCRs.[11] Most compelling, the discovery that β-arrestins (arrestin 2 and 3) function as alternative transducers of GPCR signals has challenged the basic concept of GPCR signaling.[11,52-53] Originally regarded as mediators of GPCR desensitization (through internalization into clathrin-coated pits),[54-55] β-arrestins are ubiquitously expressed cellular regulatory proteins that are now recognized as true adapter proteins that transduce signals to multiple effector pathways such as MAPKs, SRC, nuclear factor κB (Nf-κB) and phosphatidylinositol 3-kinase (PI3K).[56] Since arrestin binding uncouples GPCRs

from G-proteins, arrestin-dependent signals may represent a form of second wave signaling by desensitized receptors.

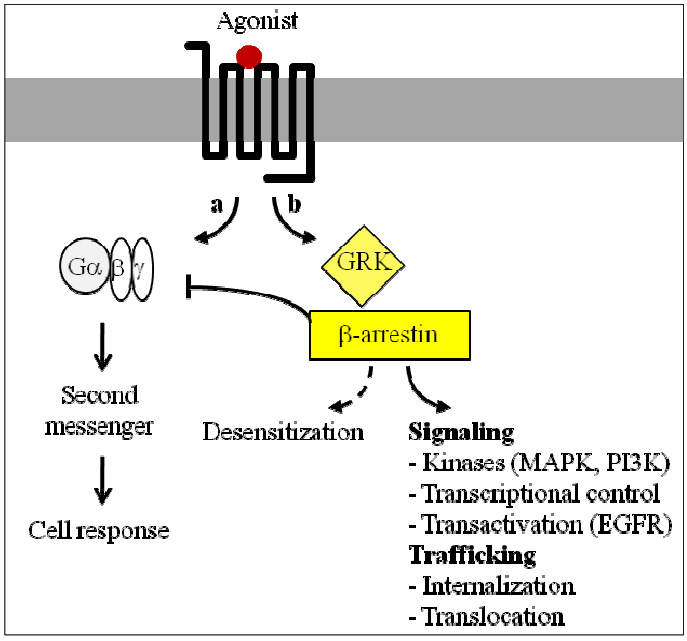


Figure 1.3. G-protein and β-arrestin mediated signaling. Classical model: signaling is mediated by G-proteins, followed by phosphorylation by GRK, and desensitization is mediated by β-arrestins. Current model: Binding of a ligand results in signaling by G-proteins and/or β-arrestins, as well as desensitization and internalization by β-arrestins. In a system with functional selectivity, signaling mainly proceeds through one pathway (**a** *vs.* **b**). According to Rajagopal et al.[56]

Nowadays, it is apparent that different ligands can differently bias GPCR conformations towards one type of behavior versus another.[37] The selective stimulation of some but not all possible signaling pathways has been postulated as "functional selectivity",[57] also known as "biased agonism"[58] or differential receptor-linked effector actions.[59-60] For GPCRs, these aspects may include signaling via multiple G-protein regulated pathways, including pathways regulated by either Gα or Gβγ subunits, as well as engaging mechanism involved in receptor desensitization (phosphorylation of the receptor, binding to arrestin, internalization) and arrestin mediated signaling.[61] By now, functionally selective ligands for G-protein mediated or arrestin mediated processes are known for many GPCRs,[62] including μ-opioid,[63-65] serotonin 5-HT_{2A},[66-68] β-adrenergic,[69-72] angiotensin II AT_1,[73-75] dopamine D_2[76-79] and histamine H_1 receptors.[80] Such biased ligands are not only useful tools to investigate GPCR signaling, but might also harbor an improved potential as fine-tuned therapeutics.[81] Besides, allosteric ligands, which could modulate the signaling cascades and biochemical responses triggered by endogenous ligands, can also impose biased agonism and therefore hold promises for future pharmacology.[82]

1.1.4 GPCR oligomerization and bivalent ligands

GPCRs have classically been assumed to exist and function as monomeric entities in a 1:1:1 stoichiometry with the G-protein and the ligand. But over the last few decades the understanding of GPCR structure and function has been challenged by the discovery that GPCRs are able to form homo- and hetero-oligomeric complexes.[83-85] Evidence of GPCR dimerization is provided by biochemical, biophysical and functional studies, for instance, by cross-linking, immunoblotting, co-immunoprecipitation and atomic force microscopy as well as fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET). The latter have been used to substantiate the occurrence of GPCR dimerization in living cells.[84,86] For receptors, such as the tyrosine-kinase and the steroid-hormone receptor, constitutive or ligand-induced oligomerization has long been known as essential for signaling.[87] Meanwhile, the existence of homodimers has also been demonstrated for several class A and C GPCRs including dopamine D_2 and D_3 receptors,[88-89] the β_2-adrenoceptor,[90] the 5-HT_{1D} serotonin receptor,[88] the histamine receptor subtypes,[91-95] opioid receptors,[96-98] the mGluRs[99-100] and the Ca^{2+}-sensing receptor.[101] Besides homodimerization, there is growing evidence that heterodimerization can result in receptor complexes that have ligand-binding and signaling properties distinct from their constituent monomers.[83] Distinct characteristics arising from hetero-dimerization have been demonstrated for the κ- and δ-opioid receptors,[98] the μ- and δ-opioid receptors[102] or the angiotensin AT_1 and bradykinin B_2 receptors.[103] For class C GPCRs dimerization is essential for function, with the association of two identical or two distinct monomers being required to get a functional receptor, for example, $GABA_{B1}$/$GABA_{B2}$ is known as an obligate heterodimer.[104] Although few is known about the physiological role of GPCR dimerization, recent findings indicate a pivotal role in receptor trafficking, signaling, pharmacology and internalization.[84,105] Three sites could be involved in receptor-receptor interactions of GPCRs: extracellular loops, transmembrane helices and intracellular loops. These regions can interact via covalent bonds (e.g. disulfide bonds), non-covalent interactions (e.g. hydrophobic interactions between TM helices or coiled coil structures) or a combination of both. While for the majority of class C receptors, an intermolecular disulfide bond between the amino termini has been shown to be crucial,[83] for class A receptors, the TM helices 1 and 4-6 are thought to be involved in oligomerization.[106-108]

Provided that oligomeric GPCRs have biological functions, oligomeric entities offer new opportunities for drug design by exploiting multivalency. Usually, the term “bivalent ligands” refers to molecules containing two sets of pharmacophoric entities linked through a spacer. However, in the broader sense bivalent ligands can be divided in molecules containing two sets of pharmacophoric groups or a single pharmacophore connected to a non-pharmacophoric recognition unit.[109] The design of bivalent ligands requires the consideration of various general features including a suitable monomeric lead compound, an appropriate attachment point of the spacer and a spacer with suitable length and chemical composition.[110-111] Different binding modes of bivalent ligands at the receptor(s) are imaginable (Figure 1.4). If the spacer is of sufficient length the ligand may bridge two neighboring receptors, each pharmacophoric moiety simultaneously interacting with the recognition sites of both protomers. For bivalent ligands with shorter linkers an accessory recognition site next to the orthosteric binding site of a single protomer is probable. In both cases, the ligand first binds in a univalent manner to the receptor. Thereby, the second pharmacophoric moiety of the bivalent ligand is arranged in closer proximity to the second binding site (neighboring protomer or accessory binding site) corresponding to a high local concentration of the second recognition unit. Bivalent ligands are thought to exhibit a greater potency than that corresponding to double concentration of a monovalent ligand.[85,109] This concept has been studied for many GPCRs, for instance, for opioid receptors in more detail.[112] The bivalent ligand approach has proven to be promising to improve not only potency and selectivity but also the pharmacokinetic profile of compounds.[110,113-114]

There is evidence that GPCRs can form homo- and heterodimers, yet many of the most potent bivalent ligands have relatively short linking groups, suggesting that the compounds interact with neighboring binding sites on a single receptor (cf. Fig. 1.4 **A**).[113-114] This mechanism fits to the message-address concept proposed by Schwyzer,[115] in which the pharmacophore can be considered as the “message” that is recognized by a family of receptors and the second (non-)pharmacophoric entity is considered as the “address” conferring additional affinity. Another explanation to account for differences between monomeric and bivalent ligands involves the induction and stabilization of receptor dimerization, as dimerization plays an important role in the function of GPCRs.[113] Finally, the affinity of bivalent ligands can also be influenced by cooperative

effects.[85,109,116] For instance, the phenomenon that binding of one pharmacophoric moiety facilitates the binding of the second pharmacophore is termed positive cooperativity.[82,116]

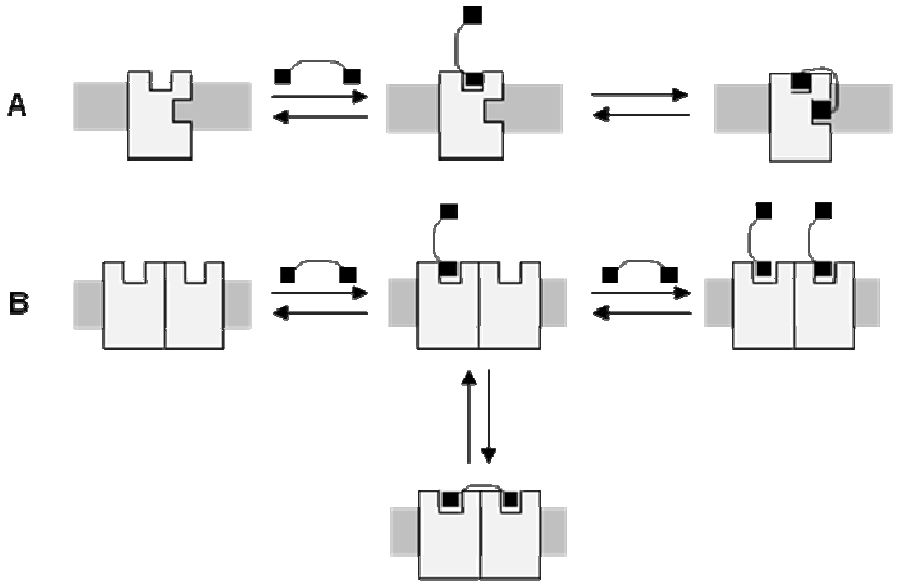


Figure 1.4. Bivalent ligand binding to **A**, a GPCR with an accessory binding site, or to **B**, a GPCR dimer. The bivalent ligand is believed to bind in a univalent manner before addressing the second binding site. According to Portoghese et al.[85,117]

Taken together, the bivalent ligand approach is a valuable strategy of modern medicinal chemistry to obtain highly potent and selective compounds, but, unfortunately, there is no universal recipe for success to improve pharmacological or drug-like properties of compounds upon dimerization. Regardless of their potential therapeutic values, bivalent ligands are required as pharmacological tools to expand the knowledge of structure-activity relationships, to explore the ligand-receptor interactions and, possibly, to investigate the functional relevance of receptor dimerization. The application of the bivalent ligand approach to histamine H_2 receptor agonists is subject of this work (cf. chapters 4 and 5).

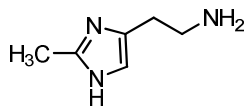
1.2 Histamine receptors

Histamine exerts its effects through the interaction with four histamine receptor subtypes, designated H_1 (H_1R), H_2 (H_2R), H_3 (H_3R) and H_4 (H_4R) receptors, all of which belong to the class A of GPCRs.[118-120] Long before cloning of their respective genes,[121-123] the H_1R and the H_2R were pharmacologically identified and they have been targets of blockbuster

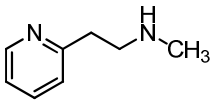
drugs for decades. While H_1R antagonists ("antihistamines") are well established in the treatment of allergic disorders, H_2R antagonists have been used as antiulcer drugs ("H_2R blockers").[118] The identification of the presynaptic H_3R as a new receptor subtype[124-125] gave rise to a new field of interest. The H_3R is now regarded as a general regulatory system in the CNS and a potential target for new therapeutics.[126] More recently, the use of genomic databases resulted in the identification of the fourth histamine receptor due to its homology with the H_3R.[127-128] The average sequence homology between the HR subtypes is relatively low (20 %) except for H_3R and H_4R, which share overall sequence homology as high as 37 %.[119]

The histamine **H_1R** is mainly expressed on smooth muscle cells, endothelial cells, cells of the immune system and the CNS.[129] The human receptor represents a 487 amino acid protein that preferentially couples to the pertussis-toxin insensitive $G_{q/11}$-protein. Its stimulation affects the inositol phospholipid signaling system, resulting in the formation of IP_3 and DAG (as explained in chapter 1.1.3.1), which yields in Ca^{2+}-mobilization from intracellular stores and activation of protein kinase C.[129-130] Most effort has been directed towards the development of H_1R antagonists, whereas H_1R agonists are useful as pharmacological tools rather than as drugs. The only H_1R agonist used in therapy is betahistine (Aquamen®) for the treatment of Menière`s disease.[131] Other H_1R agonists like the histaprodifens represent valuable pharmacological tools to analyze H_1R function in cellular and organ systems.[132-134] The first generation H_1R antagonists like mepyramine (Pyrilamine®), chlorpheniramine and promethazine (Prothazin®) have been therapeutically used for the treatment of allergic diseases since the 1940s.[135] Currently, mepyramine is the most commonly used reference H_1R antagonist for pharmacological studies. To reduce the sedative side effects, more polar antagonists that are no longer able to pass the blood brain barrier like cetirizine (Zyrtec®) and fexofenadine (Telfast®) were developed. These compounds belong to the non-sedating second generation of H_1R antagonists and are still among the top selling drugs for the treatment of allergic disorders.

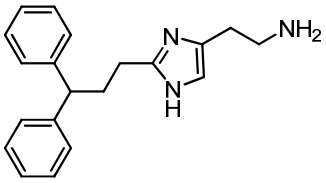
H_1R agonists



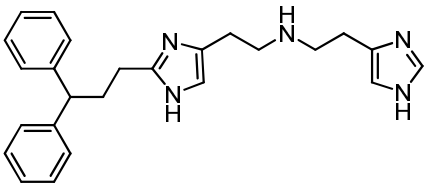
2-Methylhistamine



Betahistine

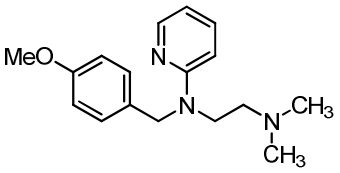


Histaprodifen

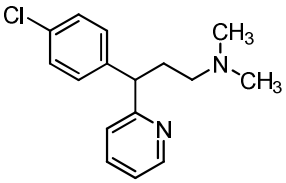


Suprahistaprodifen

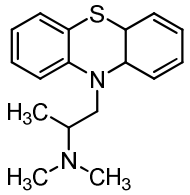
H_1R antagonists



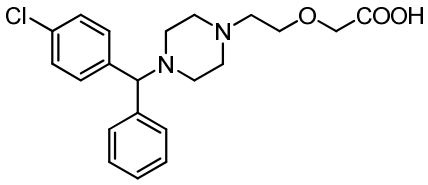
Mepyramine



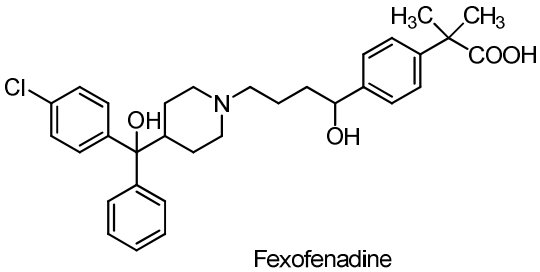
Chlorpheniramine



Promethazine



Cetirizine



Fexofenadine

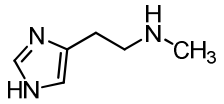
Figure 1.5. Structures of selected H_1R ligands.

A detailed description of the **H_2R** is given in chapter 1.3.

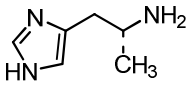
The histamine **H_3R** was discovered by Schwartz and co-workers in 1983[125] and firstly cloned in 1999.[136] The hH_3R consists of 445 amino acids and is mainly expressed in the CNS, where it acts as a presynaptic auto- and heteroreceptor controlling the release of histamine and various other neurotransmitters, including dopamine, serotonin, noradrenalin and acetylcholine.[137-138] As such, the H_3R is supposed to be involved in a multitude of CNS functions, like locomotor activity, wakefulness, food intake, thermoregulation and memory.[91] Receptor activation leads to the recruitment of $G_{i/o}$-proteins, which in turn lowers the cAMP level by inhibition of the adenylyl cyclase. In addition, a variety of other effector pathways can be activated including the activation of

MAPK, PI3K and phospholipase A_2 (PLA_2).[139-140] Up to now, no H_3R ligand is on the drug market. However, the H_3R has attracted interest as a potential drug target for the treatment of various disorders and diseases, including dementia, Alzheimer`s disease, narcolepsy, insomnia, attention deficit hyperactivity disorder, schizophrenia as well as for the treatment of myocardial ischemic arrythmias, migraine and inflammatory and gastric acid related diseases.[140-145] Therefore, H_3R agonists as well as antagonists and inverse agonists are needed and currently many compounds from different pharmaceutical companies are under clinical investigation.[146] H_3R antagonists can be divided into imidazole-containing antagonists such as thioperamide and clobenpropit and non-imidazole antagonists, for example JNJ10181457,[147] with improved drug-like properties and selectivity, in particular over the closely related H_4R. Typical H_3R agonists are N^{α}-methylhistamine and (R)-α-methylhistamine[148] as well as imetit[149] and the H_3R selective methimmepip,[142] which are structurally less related to histamine. To increase the bioavailability and CNS permeability of the very polar (R)-α-methylhistamine more lipophilic azomethine prodrugs like BP 2-94 were successfully developed.[150]

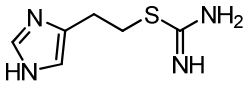
H_3R agonists



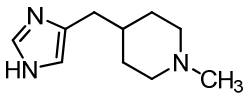
N^{α}-Methylhistamine



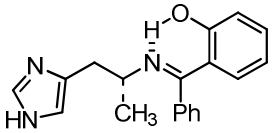
(R)-α-Methylhistamine



Imetit

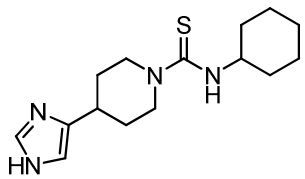


Methimmepip

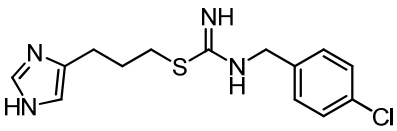


BP 2-94

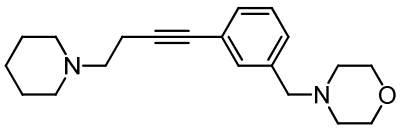
H_3R antagonists



Thioperamide



Clobenpropit



JNJ 10181457

Figure 1.6. Structures of selected H_3R ligands.

Cloning of the H_3R gene provided the basis for a fourth histamine receptor subtype.[127-128] The histamine **H_4R** is mainly expressed in various cells of the immune system like mast

cells, basophils, eosinophils, T-lymphocytes and dendritic cells[128,143] suggesting that it plays an important role in different inflammatory, autoimmune and allergic disorders.[151] Additionally, the H_4R is also expressed in the CNS.[152] The human receptor subtype consists of 390 amino acid and just as the H_3R couples to $G_{i/o}$-proteins resulting in AC inhibition and activation of MAPKs.[128,153] Little is known about the exact (patho)physiological roles of the H_4R, but the activation of H_4Rs has been shown to induce several responses closely associated to immune cells, e.g. chemotaxis, chemokine production and Ca^{2+}-mobilization in mast cells, monocytes and eosinophils.[143] Currently, drug research in the H_4R field is focused on antagonists due to the prospect of new therapies for the treatment of inflammatory diseases. The blockade of the receptor by antagonists is considered a promising approach for the treatment of diseases like purities, asthma, inflammatory bowel disease or rheumatoid arthritis.[154] The supposed role of the H_4R in immunological responses overlaps with the function of the H_1R, suggesting that combined H_1- and H_4-receptor ligands might be beneficial for the treatment of inflammatory diseases. On the other hand, selective agonists definitely represent valuable pharmacological tools for further investigations on the biological role of the H_4R. Due to the high homology with the H_3R, many H_3R ligands also bind to the H_4R, albeit with a different rank order of affinity and potency. In search for selective ligands for the latest histamine receptor subtype, many GPCR ligands were pharmacologically studied resulting in the identification of numerous ligands from different structural classes.[143] The first selective H_4R agonists were OUP-16, a chiral tetrahydrofuran analog,[155] and later 5-methylhistamine (also referred to as 4-methylhistamine), which was originally considered as a selective H_2R agonist. Very recently, highly potent and selective cyanoguanidine-type H_4R agonists such as UR-PI376 were successfully developed in our working group.[117,143,156] Interestingly, thioperamide is not only an inverse H_3R agonist, but also acts as a highly active inverse H_4R agonist.[128,153,157] Meanwhile, selective H_4R antagonists such as the indole-2-carboxamide JNJ7777120[158] and different 2-aminopyrimidines[159] have been developed. Most notably, JNJ7777120 is a valuable pharmacological tool and has already been employed in several animal models to study the biological function of the H_4R.[160-162] However, the investigation of the biological role of the H_4R in animal models is hampered by species-dependent discrepancies regarding receptor selectivity, potencies and even by opposite qualities of action of the available pharmacological tools.[143,163]

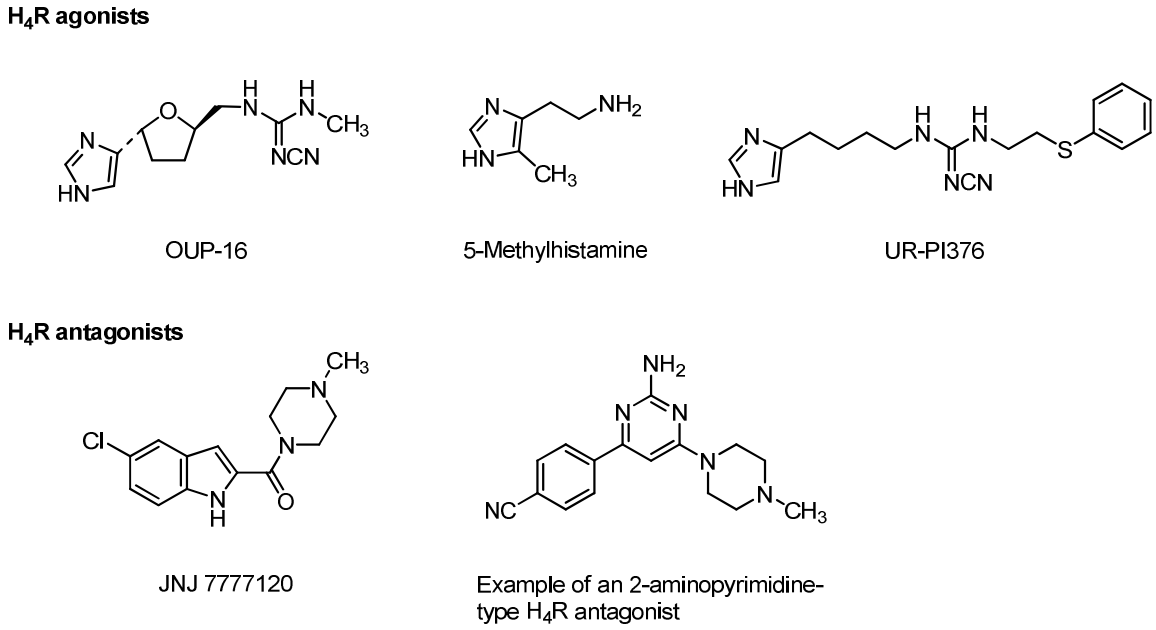


Figure 1.7. Structures of selected H_4R ligands.

1.3 The histamine H_2 receptor and its ligands

The histamine H_2R was pharmacologically characterized by Black et al. in 1972 using the first H_2R antagonist burimamide.[164] Contrary to the classical antihistamines, burimamide was able to block the histamine mediated gastric acid secretion and positive chronotropic effect on the heart. In 1991, Gantz and coworkers cloned human and canine H_2Rs.[122-123] The human H_2R consists of 359 amino acids and couples to the G_s-protein, resulting in increased cAMP levels via activation of the adenylyl cyclase.[118,165-166] As explained in section 1.1.3.1, cAMP can activate protein kinases which phosphorylate regulatory proteins, leading, for instance, to an influx and intracellular mobilization of Ca^{2+} in cardiac myocytes (Figure 1.8). Besides the phosphodiesterase-catalyzed inactivation of cAMP, the cAMP response attenuates after minutes due to agonists-mediated receptor desensitization and internalization of the receptor.[167-168] It is demonstrated that β-arrestin, dynamin (a 100 kDa GTPase) and clathrin are involved in H_2R internalization and its rapid recycling to the cell surface.[167] In several systems, the H_2R also couples to the G_q-protein resulting in PLC stimulation.[169-170] Thus, the activity of the H_2R results from a regulated balance among the diverse mechanism of receptor signaling and trafficking.

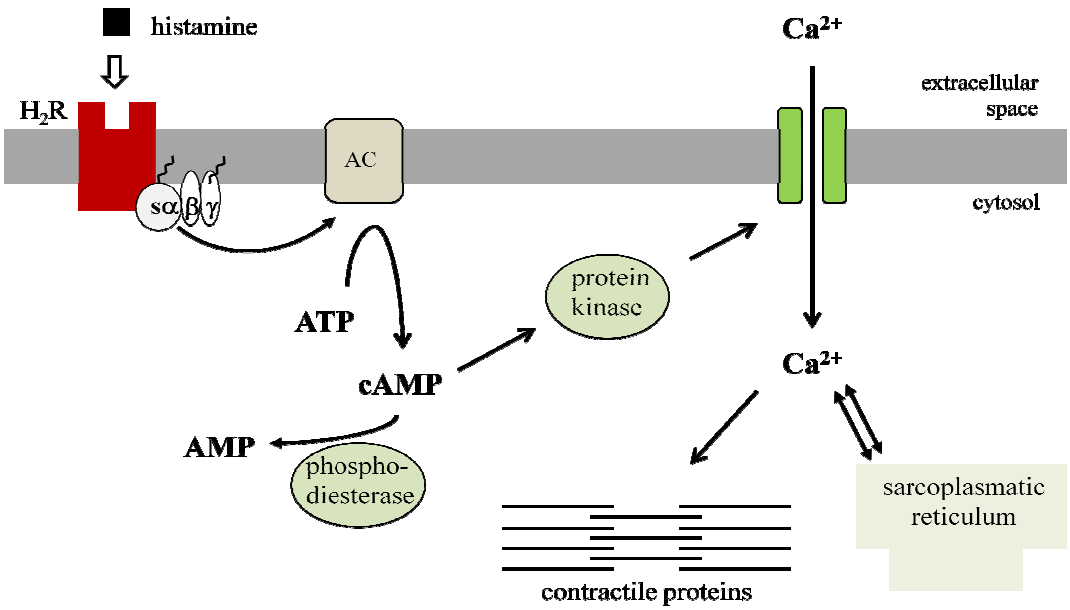


Figure 1.8. H_2R mediated signaling; Cardiac myocyte as example. According to Del Valle et al.[165]

H_2Rs are located on gastric parietal cells and several other tissues and cells including leukocytes, airways, heart, uterus vascular smooth muscles and the brain.[118,171-172] An essential physiological function of the H_2R is the control of gastric acid secretion from parietal cells.[164] Activation of cardiac H_2Rs mediates positive chronotropic and inotropic effects,[173] and histamine-mediated smooth muscle relaxation has been documented in airways, uterus and blood vessels.[174] Moreover, promyelocytic leukemic cells express the H_2R and its activation triggers the functional differentiation to mature granulozytes.[175-176] H_2Rs are also reported to have numerous functions in the immune system. For example, H_2R have been shown to block the histamine release from mast cells, to inhibit T-cell modulation and to modulate the production of cytokines.[177-178] In addition, audioradiographic mapping of the brain with the high affinity H_2R radioligand [^{125}I]iodoaminopotentidine revealed highest densities in the basal ganglia, hippocampus, amygdale and cerebral cortex.[171] So far, the function of H_2Rs in the CNS has not been identified.[135] Although, the CNS permeability was already demonstrated for the H_2R antagonist zolantidine,[179] most of the therapeutically used H_2R ligands do not cross the blood-brain barrier in significant amounts. Therefore, centrally active H_2R ligands are promising pharmacological tools to study the role of these receptors in the CNS.

To date, numerous H_2R agonists and antagonists have been identified, with the guinea pig atrium being the pharmacological standard *in vitro* model for ligand characterization for

decades.[118] The search for H_2R antagonists as drugs for the treatment of gastric and duodenal ulcer started with burimamide,[164] the first selective H_2R antagonist and resulted in the development of cimetidine (Tagamet®) and its introduction into the clinic about 35 years ago. Very fast, cimetidine and other H_2R blockers such as famotidine (Pepdul®) and ranitidine (Zantic®) became blockbuster drugs.[118,180] In addition to the marketed drugs (in Germany: cimetidine, ranitidine, nizatidine, famotidine, roxatidine acetate), numerous structurally diverse highly active H_2R antagonists are known, for example, tiotidine and aminopotentidine, which are used as pharmacological tools. Very recently, a new series of H_2R antagonists was developed in our working group, replacing the cyanoguanidine group of potentidine-related piperidinomethylphenoxyalkylamines by squaramides. Additional coupling with ω-aminoalkyl spacers allows for labeling reactions or bivalent ligand construction (cf. UR-DE96, Fig. 1.9).[181]

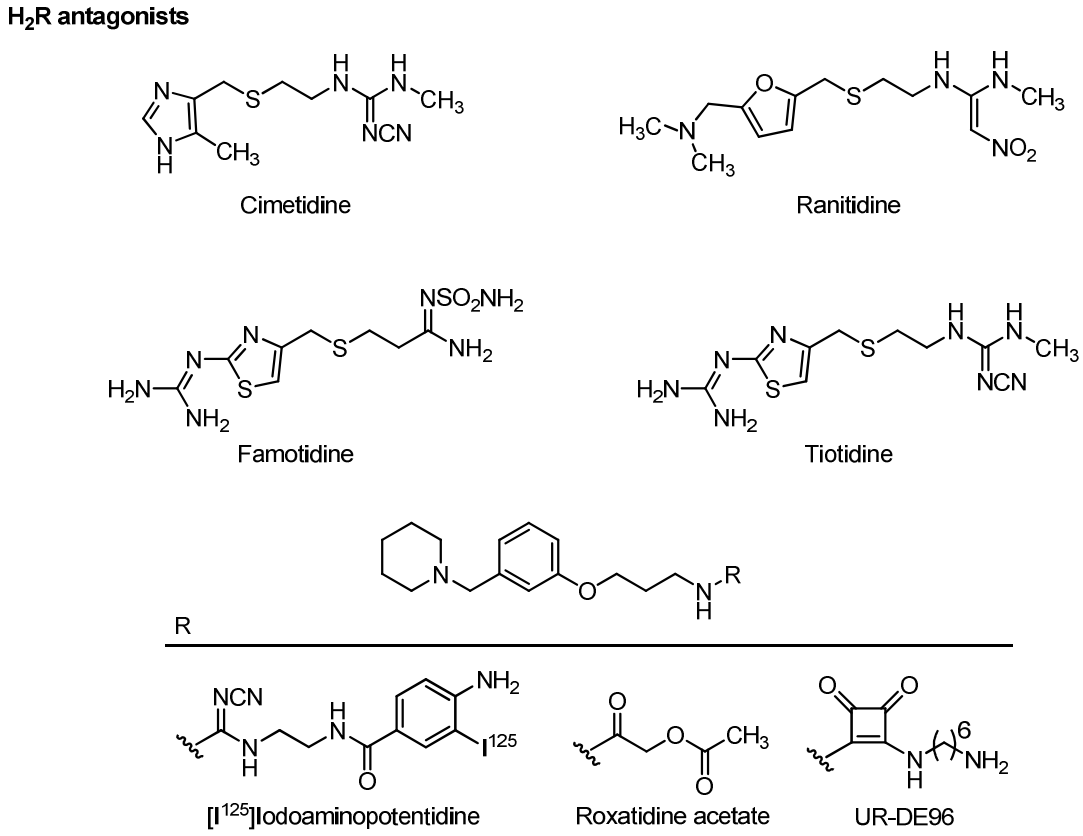


Figure 1.9. Structures of selected H_2R antagonists.

Whereas H_2R antagonists became standard drugs for the treatment of gastric and duodenal ulcers,[180,182] H_2R agonists are mainly used as pharmacological tools to study the physiological and pathophysiological role of this histamine receptor. Nevertheless, H_2R agonists are of potential therapeutic value as positive inotropic vasodilators for the

treatment of acute congestive heart failure,[183] as anti-inflammatory agents,[184-185] or as differentiation-inducing agents in acute myelogenous leukemia (AML).[176] Actually, histamine dihydrochloride (Ceplene®) is administered in conjunction with low doses of immune-activating cytokine interleukin-2 (IL-2) in the post-remission phase of AML. Given that the effect of histamine is mediated via the H_2R, new selective H_2R agonists with suitable pharmacokinetic properties for *in vivo* applications are promising drug candidates. Compared to the amine-type H_2R agonists (histamine, dimaprit, amthamine), guanidine-type compounds (impromidine,[186-187] arpromidine[188-190]) are much more potent. At the guinea pig right atrium, these compounds show up to 400 times the potency of histamine. The binding site of histamine in the H_2R was identified by molecular modeling approaches and *in vitro* mutagenesis studies. Hence, histamine probably binds in its N^{π}-tautomeric form to the receptor by forming H-bonds with Asp-186 and Tyr-182 in TM5 and the protonated primary amino group interacts with the highly conserved Asp-98 in TM3 (cf. Figure 1.11 **B**).[191-192] As an alternative to Tyr-182, Thr-190 is discussed to participate in ligand binding.[193-194] The interaction of guanidine-type agonists may be interpreted by analogy with this model: the strongly basic guanidino group (pKa ≈ 13), considered a mimic of the primary amino group in histamine, is essential for the H_2R agonistic activity of guanidine-type compounds, but is also responsible for very low oral bioavailability and lack of CNS penetration.[191] In principle, this problem can be solved by prodrug strategies as demonstrated by the introduction of alkoxycarbonyl groups at the guanidine group.[195] Though, such derivatives were not active until ester cleavage and decarboxylation, and centrally active H_2R agonists could not be obtained following this approach.

Major progress in the development of orally active non-prodrug H_2R agonists was achieved with the bioisosteric exchange of the guanidine by an acylguanidine moiety, resulting in N^{G}-acylated imidazolylpropylguanidines (e.g. UR-AK24, Fig. 1.10), a new class of potent H_2R agonists with substantially reduced basicity (by 4-5 orders of magnitude). *In vivo* studies confirmed that the reduced basicity results in absorption from the gastrointestinal tract and penetration across the blood brain barrier.[191] Unfortunately, the selectivity of N^{G}-acylated imidazolylpropylguanidines for the H_2R turned out to be poor, in particular versus H_3R and H_4R. This drawback appears to depend on the "privileged" imidazole moiety. Therefore, the bioisosteric replacement of the imidazole ring is the key to improve the selectivity for the H_2R. The 2-amino-4-methylthiazol-5-yl

moiety is a bioisostere of the imidazole ring in the moderately potent H_2R-selective amthamine, a thiazole analog of histamine and a cyclic analog of dimaprit. Amthamine is a full H_2R agonist with slightly higher potency than histamine at the isolated guinea pig right atrium[196-197] and most notably, it is devoid of histamine H_1R, H_3R and H_4R stimulatory activities at relevant concentrations.[198-199] Very recently, supported by docking studies (cf. Figure 1.11 **A**), this bioisosteric approach was successfully applied to acylguanidine-type H_2R agonists. The bioisosteric replacement of the imidazole ring in N^G-acylated imidazolylpropylguanidines by a 2-aminothiazol-5-yl moiety resulted in potent H_2R agonists with much greater selectivity for the human H_2R over H_3 and H_4 receptors.[192] Thus, N^G-acylated aminothiazolylpropylguanidines (e.g. UR-PG278, Fig. 1.10) combine the high selectivity for the H_2R with improved pharmacokinetic properties, resulting in valuable pharmacological tools to evaluate the physiological role of H_2Rs, for instance, in the CNS, and are promising starting points for the development of compounds suitable for *in vivo* application.

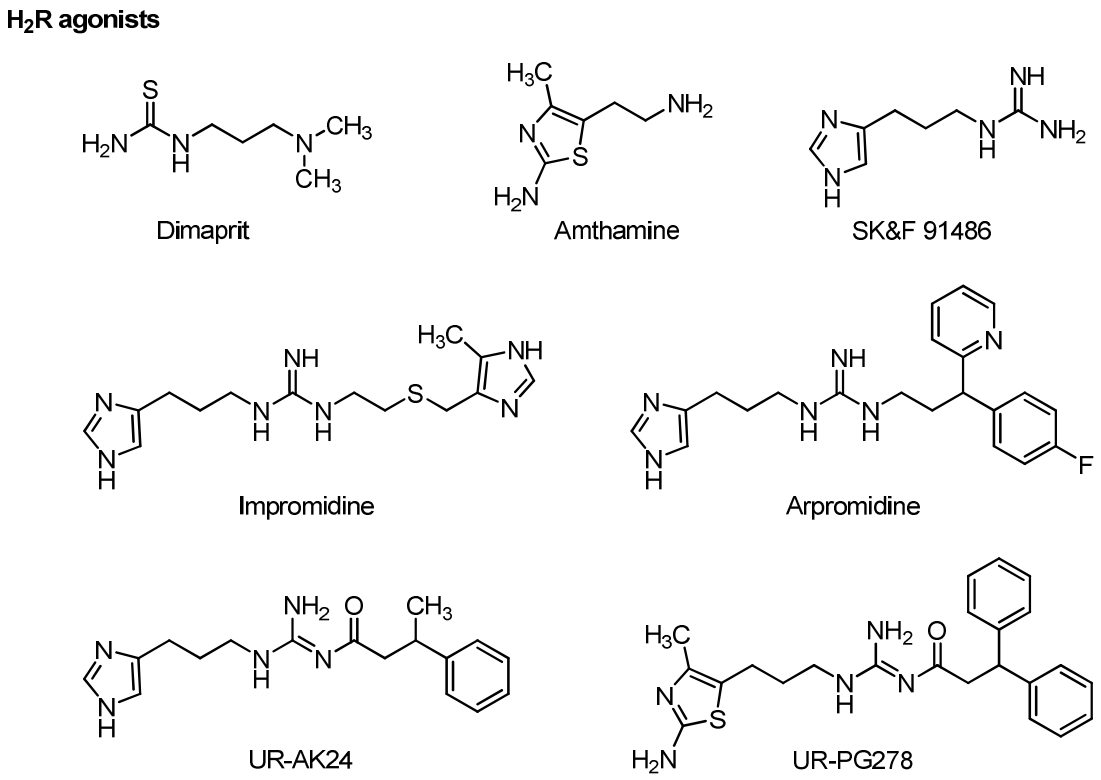


Figure 1.10. Structures of selected H_2R agonists.

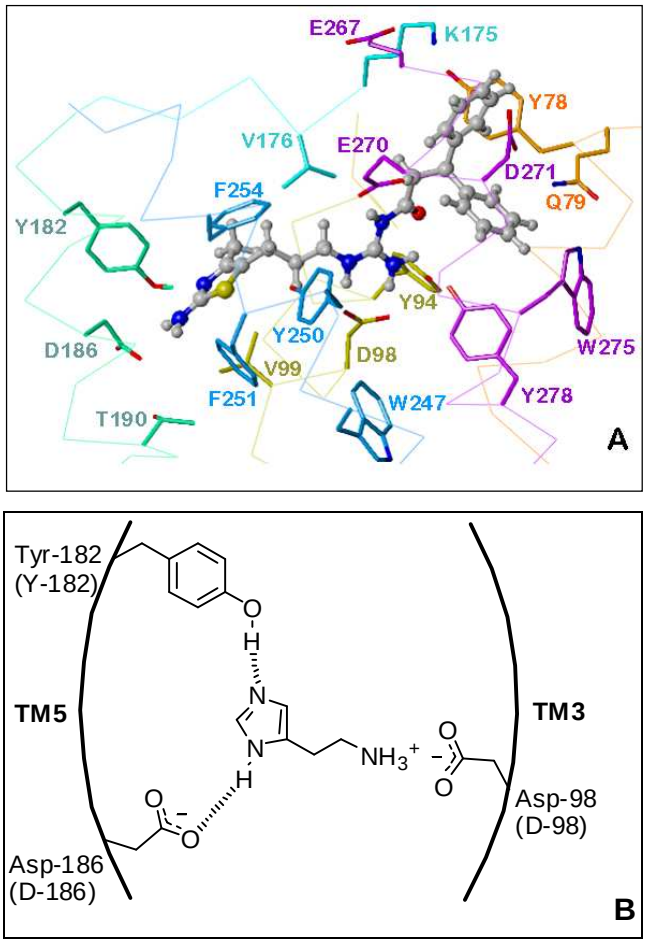


Figure 1.11. A: Model of the gpH_2R binding site for UR-PG278 with illustration of side chains and C_α atoms of all amino acids within 3 Å around the ligand and, additionally, the putative toggle switch Trp-247. The backbone and the C atoms of the amino acids are individually drawn in spectral colors: TM2 – orange, TM3 – yellow, e2 – cyan, TM5 – greenblue, TM6 – blue, TM7 – purple. All nitrogens – blue, oxygens – red, C and H atoms of the ligand – grey, C_α trace – lines, binding site C_α atoms and side chains – sticks, ligand – balls and sticks. Adapted from Kraus et al[192] with permission from John Wiley and Sons, copyright 2009. **B:** Proposed binding mode of histamine at the H_2R.

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Chapter 2

Scope and objectives

Although numerous compounds were described as histamine H_2 receptor (H_2R) agonists decades ago, after discovery of the histamine H_3 (H_3R) and H_4 receptors (H_4R), the H_2R selectivity of compounds such as 5-methylhistamine,[1] dimaprit,[2] impromidine[3] or arpromidine[4] turned out to be comprised.[5-7] For instance, 5-methylhistamine is nowadays considered as selective for the H_4R. Thus, new selective H_2R agonists are needed as pharmacological tools to explore the (patho)physiological role of the H_2R and as potential drug candidates, for instance, for the treatment of acute myelogenous leukemia. Recently, in search for H_2R agonists derived from guanidine-type compounds, N^G-acylated hetarylpropylguanidines were discovered in our laboratory as a new class of potent H_2R agonists with considerably reduced basicity.[6-7] Lowering the basicity resulted in improved pharmacokinetic properties such as oral bioavailability and CNS penetration.[6] Moreover, these acylguanidines proved to be highly selective for the H_2R, when the imidazole ring was replaced with a bioisosteric amino(methyl)thiazole moiety.[7]

Based on these preceding proof-of-concept studies, this thesis aimed at novel N^G-acylated 3-(2-aminothiazol-5-yl)propylguanidines as potent and selective H_2R agonists, which might be useful as pharmacological tools to evaluate the physiological role of H_2Rs, for instance, in the CNS. The structure-activity relationships, the selectivity profiles and the contribution of the 4-methyl substituent in the thiazole ring should be discussed.

As ligands containing two pharmacophoric entities should possess increased H_2R agonistic potency[8-10] and might be useful to investigate GPCR dimerization,[11-12] the feasibility of the bivalent ligand approach to acylguanidine-type H_2R agonists was intended to be evaluated by linking two hetarylpropylguanidines through dicarboxylic acids of different structure and length. In continuation to this approach, unsymmetrical

bivalent compounds bearing two different pharmacophoric moieties had to be designed, synthesized and pharmacologically investigated in order to elaborate structure-activity relationships with respect to the role and the interaction site of the second set of pharmacophoric groups. Herein, bivalent compounds with combined agonistic and antagonistic pharmacophores should be considered.

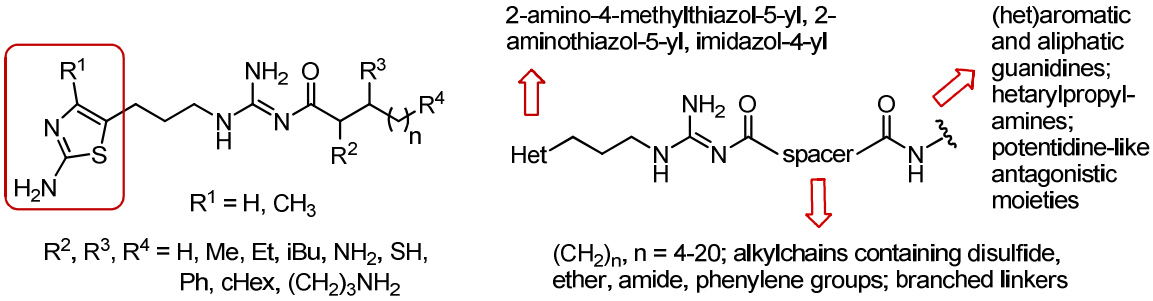

Figure 2.1. General structure of mono- and bivalent acylguanidine-type H_2R agonists.

In addition, as a prerequisite for the application of acylguanidine-type H_2R agonists as pharmacological tools in cell based *in vitro* studies or future *in vivo* experiments, representative compounds should be examined with respect to their drug-like properties and toxic effects. For this purpose, selected compounds had to be investigated for hemolytic activity, cytotoxicity and plasma protein binding.

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Chapter 3

N^G-Acylated 3-(2-aminothiazol-5-yl)propyl-guanidines: towards selective histamine H_2 receptor agonists

3.1 Introduction

N^G-Acylated imidazolylpropylguanidines (e.g. UR-AK24) developed in our workgroup are potent histamine H_2R agonists, but lacking selectivity for the H_2R, in particular versus H_3 and H_4 receptors.[1-3] Very recently, the bioisosteric replacement of the imidazole ring in the "privileged" imidazolylpropylguanidine moiety of acylguanidine-type H_2R agonists by a 2-aminothiazol-5-yl group resulted in almost equipotent H_2R agonists with much greater selectivity for the human H_2R over H_3 and H_4 receptors.[4] Based on these preceding proof-of-concept studies, the bioisosteric approach was continued, aiming at N^G-acylated 3-(2-aminothiazol-5-yl)propylguanidines as potent and selective H_2R agonists, which might be useful as pharmacological tools to evaluate the physiological role of H_2Rs, for instance, in the CNS. The structure-activity relationships (SAR), the selectivity profiles and the contribution of the 4-methyl substituent in the thiazole ring will be discussed.

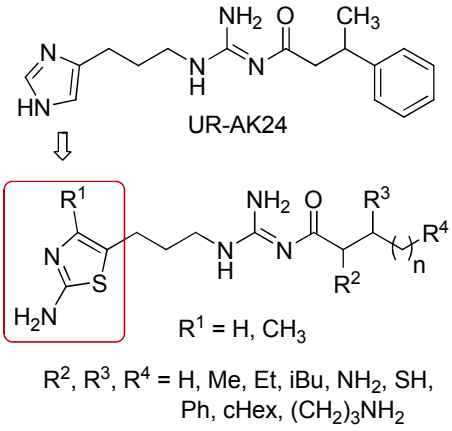
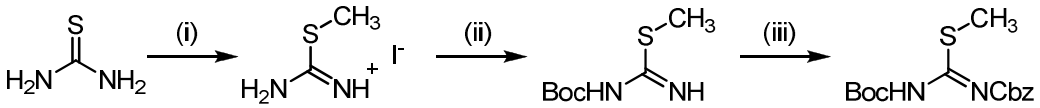


Figure 3.1. Bioisosteric replacement of the imidazole ring in N^G-aclyated imidazolyl-propylguanidines (e.g. UR-AK24) resulting in the title compounds with 2-aminothiazol-5-yl moiety.

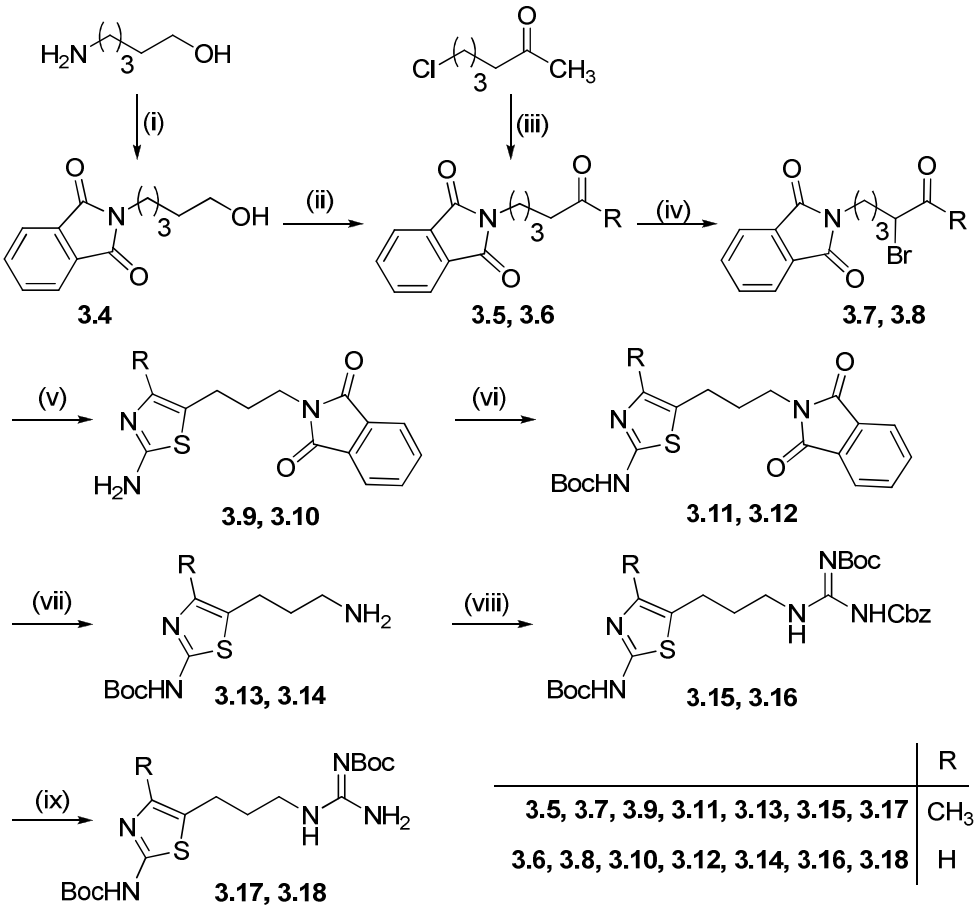
3.2 Chemistry

The preparation of the title compounds was preferentially performed according to the recently published procedures.[4] The thiazolylpropylamines **3.13** and **3.14** were synthesized from thiourea and *N*-protected α-halo-ω-amino ketone **3.7** or aldehyde **3.8**, respectively (Scheme 3.2). The amines **3.13** and **3.14** were treated with the isothiourea derivative **3.3**,[5-7] a well established guanidinylating reagent, in the presence of $HgCl_2$. Hereby, the metal ion acts as a desulfurizing agent *via* complex formation.[8-9] After hydrogenolytic cleavage of the Cbz-protecting group, the Boc-protected aminothiazolyl-propylguanidine building blocks **3.17** and **3.18** were ready for N^G-acylation, which can be achieved by the aid of peptide coupling reagents, such as EDAC or CDI, or using anhydrides, acid chlorides and active esters.



3.1 **3.2** **3.3**

Scheme 3.1. Synthesis of *N-tert*-butoxycarbonyl-*N´*-benzyloxycarbonyl-S-methylisothiourea (**3.3**). Reagents and conditions: (i) MeI (1 eq), MeOH, 1h, reflux; (ii) $(Boc)_2O$ (1 eq), NEt_3 (1 eq), DCM/abs, overnight, rt; (iii) CbzOSu (1 eq), DCM/abs, 20 h, rt.

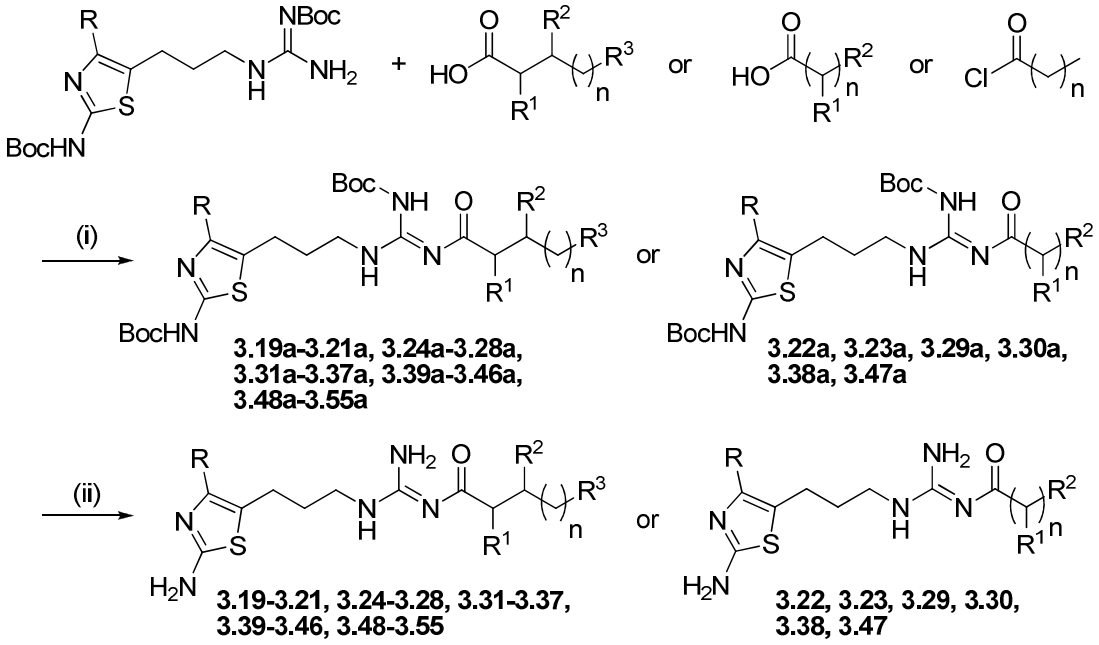


Scheme 3.2. General procedure for the preparation of the Boc-protected aminothiazolylpropylguanidines **3.17** and **3.18**. Reagents and conditions: (i) phthalic anhydride (1 eq), 3 h, 80-100 °C; (ii) $(COCl)_2$ (1.25 eq), DMSO (2.65 eq), NEt_3 (5.5 eq), DCM/abs, -50 °C, 45 min; (iii) phthalimide (0.5 eq), K_2CO_3 (0.75 eq), DMF, 24 h, 80 °C; (iv) Br_2 (1 eq), dioxane, DCM/abs, 1 h, rt; (v) thiourea (1 eq), DMF, 3 h, 100 °C; (vi) $(Boc)_2O$ (1.08 eq), NEt_3 (1.16 eq), DMAP (cat.), $CHCl_3$, overnight, rt; (vii) $N_2H_4 \cdot H_2O$ (5 eq), EtOH, overnight, rt; (viii) **3.3** (1 eq), $HgCl_2$ (2 eq), NEt_3 (3 eq), DCM/abs, 48 h, rt; (ix) H_2, Pd/C (10 %), MeOH/THF (1:1), 8 bar, 3-4 d, rt.

The synthetic strategies aimed at compounds of high purity on the low mg scale rather than at optimization of yields and synthetic routes. In this study, the guanidine building blocks **3.17** and **3.18** were coupled to commercially available and recently synthesized carboxylic acids using EDAC, HOBt and DIEA as coupling reagents as well as to pentanoyl and nonanoyl chloride. The resulting N^G-acylated di-Boc-protected aminothiazolylpropylguanidines **3.19a-3.55a** were deprotected using trifluoroacetic acid and purified by preparative RP-HPLC to yield the acylguanidines **3.19-3.55** as TFA salts with purities > 95 %.

The required carboxylic acids were mainly synthesized from commercially available or synthesized ketons according to standard procedures, including Horner-Wadsworth-

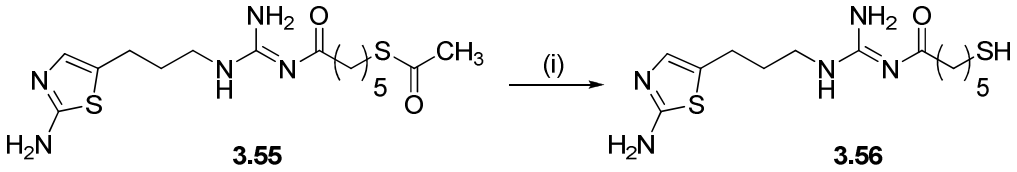
Emmons reaction with triethyl phosphonoacetate and hydrogenation of benzene rings over Rh/Al_2O_3 or Rh/C catalyst.[1,4,10-11]



Compd.	R	R^1	R^2	R^3	n	Compd.	R	R^1	R^2	R^3	n
3.19a, 319	CH_3	H	H	H	0	**3.38a, 3.38**	H	Ph	cHex	-	1
3.20a, 3.20	CH_3	H	H	CH_3	1	**3.39a, 3.39**	H	CH_3	H	Ph	0
3.21a, 3.21	CH_3	H	H	CH_3	5	**3.40a, 3.40**	H	CH_2CH_3	H	Ph	0
3.22a, 3.22	CH_3	-	Ph	-	0	**3.41a, 3.41**	H	H	CH_3	4-Me-Ph	0
3.23a, 3.23	CH_3	H	Ph	-	1	**3.42a, 3.42**	H	H	H	4-OH-Ph	0
3.24a, 3.24	CH_3	H	H	Ph	0	**3.43a, 3.43**	H	H	$(CH_2)_3NH_2$	Ph	0
3.25a, 3.25	CH_3	H	H	Ph	1	**3.44a, 3.44**	H	H	CH_3	Ph	1
3.26a, 3.26	CH_3	H	H	Ph	2	**3.45a, 3.45**	H	H	CH_3	3-OMe-Ph	1
3.27a, 3.27	CH_3	H	H	Ph	3	**3.46a, 3.46**	H	H	CH_3	4-OMe-Ph	1
3.28a, 3.28	CH_3	H	Ph	Ph	0	**3.47a, 3.47**	H	-	cHex	-	1
3.29a, 3.29	CH_3	-	cHex	-	0	**3.48a, 3.48**	H	H	H	cHex	0
3.30a, 3.30	CH_3	H	cHex	-	1	**3.49a, 3.49**	H	H	H	cHex	1
3.31a, 3.31	CH_3	H	H	NH_2	8	**3.50a, 3.50**	H	H	$CH_2CH(CH_3)_2$	cHex	0
3.32a, 3.32	H	H	H	H	0	**3.51a, 3.51**	H	H	CH_3	cHex	1
3.33a, 3.33	H	H	H	CH_3	1	**3.52a, 3.52**	H	H	CH_2CH_3	cHex	1
3.34a, 3.34	H	H	H	CH_3	5	**3.53a, 3.53**	H	H	H	NH_2	3
3.35a, 3.35	H	H	H	CH_3	15	**3.54a, 3.54**	H	H	H	NH_2	8
3.36a,3.36	H	H	H	Ph	1	**3.55a, 3.55**	H	H	H	$SCOCH_3$	3
3.37a, 3.37	H	H	H	Ph	2						

Scheme 3.3. General procedure for the coupling of the building blocks **3.17** and **3.18**, respectively, with various carboxylic acids. Reagents and conditions: (i) for **3.19a**, **3.22a-3.32a** and **3.35a-3.55a**: EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 16 h, rt; for **3.20a**, **3.21a**, **3.33a** and **3.34a**: pertinent acid chloride (1 eq), NEt_3 (1 eq), DCM/abs, 20 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt.

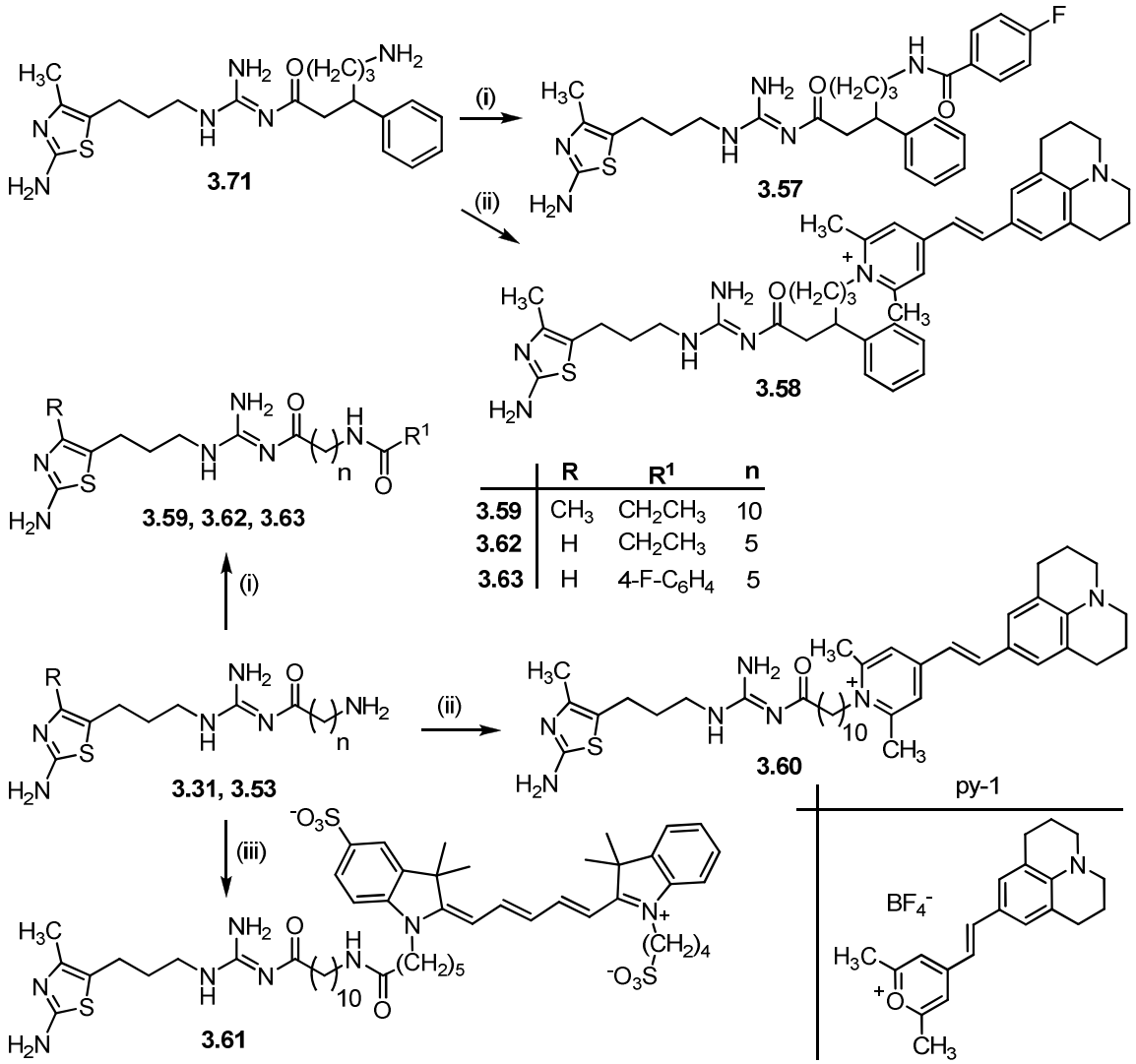
Compound **3.56** with a free thiol group was conveniently synthesized from **3.55** by cleavage of the thioester group under basic conditions followed by separation with preparative RP-HPLC.



3.55 **3.56**

Scheme 3.4. Synthesis of **3.56**. Reagents and conditions: (i) 1N NaOH, MeCN, 30 min, rt.

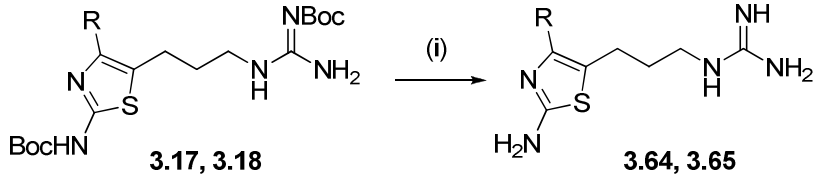
As depicted in Scheme 3.5, the free amino groups in compounds **3.31**, **3.53** and **3.71** (methylated analog of **3.43**, UR-AK466)[4,10] were acylated by stirring with the pertinent succinimidyl ester for a few hours at room temperature affording the compounds **3.57**, **3.59** and **3.61-3.63**. In addition, the fluorescent compounds **3.58** and **3.60** were synthesized from the amines **3.31** and **3.71**, respectively, and the pyrylium dye py-1 by ring transformation within one hour at room temperature. Due to the ring transformation, resulting in positively charged pyridinium compounds, the absorption maximum is shifted from about 600 nm to 500 nm.[12] This is visible by change in color from dark blue to red ("chameleon dye"). All labeled compounds were purified by preparative RP-HPLC.



	R	R^1	n
3.59	CH_3	CH_2CH_3	10
3.62	H	CH_2CH_3	5
3.63	H	4-F-C_6H_4	5

Scheme 3.5. General procedure for the preparation of compounds **3.57-3.63**. Reagents and conditions: (i) for **3.57** and **3.63**: succinimidyl 4-F-benzoate (0.8 eq), NEt_3 (3 eq), MeCN, 4-5 h, rt; for **3.59** and **3.62**: succinimidyl propionate (0.8 eq), NEt_3 (3 eq), MeCN, 4-5 h, rt; (ii) **py-1** (0.4 eq), NEt_3 (3 eq), MeCN, DMF, 1h, rt; (iii) succinimidyl ester of the cyanine dye S0586 (0.5 eq), NEt_3 (3 eq), MeCN, DMF, 20 h, rt.

Finally, deprotection of the building blocks **3.17** and **3.18** under acidic conditions resulted in 3-(2-amino-4-methylthiazol-5-ylpropyl)guanidine **3.64** and 3-(2-aminothiazol-5-ylpropyl)guanidine **3.65**, respectively.



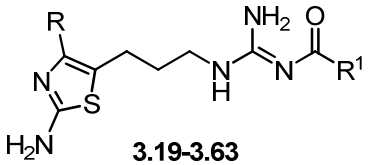
	R
3.64	CH_3
3.65	H

Scheme 3.6. Synthesis of the 2-aminothiazolylpropylguanidines **3.64** and **3.65**. Reaction and conditions: (i) 20 % TFA, DCM/abs, 3 h, rt.

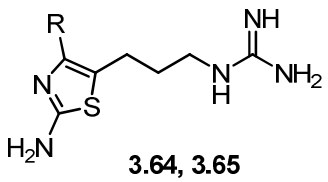
3.3 Pharmacological results and discussion

In addition to the newly synthesized N^G-acylated 3-(2-amino-4-methylthiazol-5-yl)propylguanidines **3.19-3.31** and **3.57-3.61** and N^G-acylated 3-(2-aminothiazol-5-yl)-propylguanidines **3.32-3.56**, **3.62** and **3.63**, previously prepared N^G-acylated amino-thiazolylpropylguanidines are included in this section to a more comprehensive overview of the structure-activity relationships of this class of compounds. All investigated compounds are listed in Table 3.1.

Table 3.1. Structural overview of investigated N^G-acylated aminothiazolylpropylguanidines.



3.19-3.63
3.66-3.85[a]



3.64, 3.65

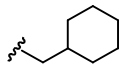
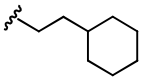
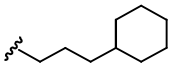
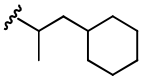
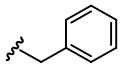
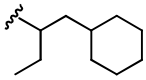
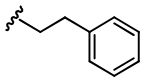
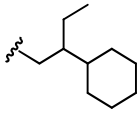
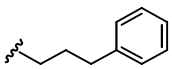
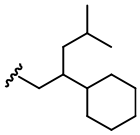
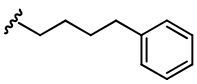
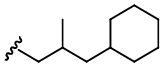
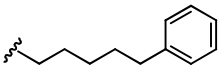
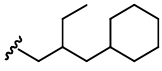
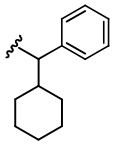
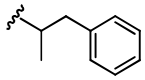
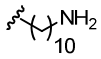
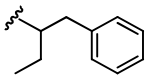
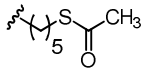
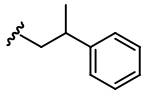
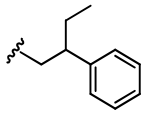
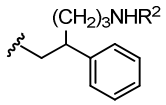
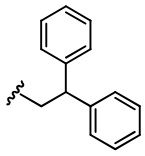
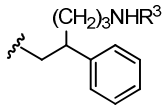
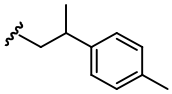
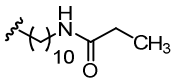
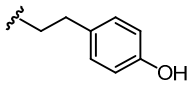
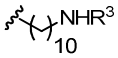
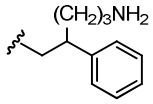
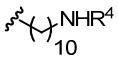
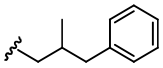
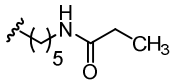
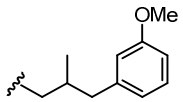
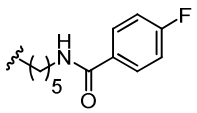
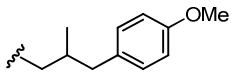
Compd.		R^1	Compd.		R^1
R = CH_3	R = H		R = CH_3	R = H	
3.19	**3.32**		**3.29**		
3.20	**3.33**		**3.30**	**3.47**	
3.21	**3.34**			**3.48**	
	3.35			**3.49**	
3.22			**3.75[a]**	**3.84[a]**	
3.23			**3.76[a]**		
3.24	**3.80[a]**		**3.77[a]**	**3.85[a]**	
3.25	**3.36**			**3.50**	
3.26	**3.37**		**3.78[a]**	**3.51**	
3.27			**3.79[a]**	**3.52**	
	3.38			**3.53**	

Table 3.1. (continued)

3.66[a]	**3.39**		**3.31**	**3.54**	
3.67[a]	**3.40**			**3.55**	
	3.81[a]			**3.56**	
3.68[a]	**3.82**[a]		**3.57**[b]		
3.28	**3.83**[a]		**3.58**[b]		
3.69[a]	**3.41**		**3.59**		
3.70[a]	**3.42**		**3.60**[b]		
3.71[a]	**3.43**		**3.61**[b]		
3.72[a]	**3.44**			**3.62**	
3.73[a]	**3.45**			**3.63**	
3.74[a]	**3.46**		**3.64**	**3.65**	-

[a] Compounds **3.66-3.85** were provided by Dr. A. Kraus. For experimental data see Ref.[4,10] [b] For full chemical structure see Scheme 3.5.

The acylguanidines, structurally related compounds (**3.64**, **3.65**) and reference substances were investigated for H_2R agonism in the steady-state GTPase assay using membrane preparations of Sf9 insect cells expressing human (h) or guinea pig (gp) H_2R-$Gs\alpha_S$ fusion proteins, measuring the enzymatic hydrolysis of radioactively labeled [γ-^{32}P]GTP or [γ-^{33}P]GTP, respectively, induced by H_2R-mediated G-protein activation (Table 3.2).[13] Additionally, selected compounds were investigated at the spontaneously beating gp right atrium as a pharmacological standard model for the characterization of H_2R ligands, determining the positive chronotropic response versus histamine as reference compound (Table 3.3).[14] To study receptor selectivity, representative compounds were investigated in GTPase assays at recombinant human histamine H_1, H_3 and H_4 receptors (Table 3.4), using membrane preparations of Sf9 insect cells expressing the hH_1R plus RGS4, the

hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ plus RGS4 and the hH_4R-RGS19 fusion protein plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$. The major advantage of the well-proven test system applied in this study is that an identical, very proximal read-out in G-protein-mediated signaling is used for any given HR subtype, namely, steady-state GTP hydrolysis. This read-out avoids bias in data interpretation caused by limited availability of downstream effectors.

3.3.1 Histamine H_2 receptor agonism

3.3.1.1 H_2R agonism at human and guinea pig H_2R fusion proteins in the GTPase assay

All investigated N^G-acylated aminothiazolylpropylguanidines proved to be partial or full agonists in the GTPase assay at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins expressed in Sf9 insect cells (Table 3.2). The most potent H_2R agonists of this series surpassed the potency of histamine about 100 and 400 times at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Comparison of the activities of **3.64** and **3.65** with the N^G-acylated compounds clearly demonstrated that the H_2R agonistic potency is strongly dependent on the structure of the acyl substituent (cf. R^1 in Table 3.1). This confirms previous observations from guanidine-type H_2R agonists revealing that the hetarylpropylguanidine part is crucial for H_2R agonism, whereas the substituent at the N^G-nitrogen is necessary as affinity-conferring moiety.[15-16]

2-Aminothiazoles lacking the 4-methyl group (**3.32-3.34**, **3.36-3.37**, **3.39-3.47**, **3.51-3.52**, **3.54**, **3.65**, **3.80** and **3.82-3.85**) showed slightly lower potencies and similar or slightly higher efficacies than their corresponding methylated analogs (**3.19-3.21**, **3.24-3.26**, **3.28**, **3.30-3.31**, **3.64**, **3.66-3.75** and **3.77-3.79**). In contrast to the thiazolylethylamine amthamine,[17] the introduction of a methyl group at position 4 of the thiazole ring did not generally increase the agonistic activity of the acylguanidine-type H_2R ligands. Thus, the methyl group is not necessary for H_2R agonistic activity. On the other hand, the 4-methyl group may be beneficial in terms of toxicity. Recently, strong evidence was arising concerning a toxic potential of 2-aminothiazoles due to bioactivation of the heterocycle resulting in electrophilic intermediates capable of binding to proteins covalently.[18-19]

In accordance to the structure-activity relationships of N^G-acylated imidazolylpropyl-guanidines,[1] the replacement of a phenyl with a cyclohexyl ring resulted mostly in higher

potencies and efficacies at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, for example, **3.29** versus **3.22**, **3.30** versus **3.23**, **3.84** versus **3.83** and **3.85** versus **3.82**. The agonistic activity was strongly affected by the chain between carbonyl group and phenyl or cyclohexyl ring, respectively: compounds with a two- to three-membered carbon chain (**3.25**, **3.26**; **3.36**, **3.37**; **3.48**, **3.49**) were most potent at both hH_2R-$Gs\alpha_S$ (pEC_{50} values $\leq$ 7.83) and gpH_2R-$Gs\alpha_S$ (pEC_{50} values $\leq$ 8.13). While methyl substituents in α- or β-position to the carbonyl group in 3-phenyl- and 3-cylohexylpropanoyl compounds were well tolerated, the introduction of more bulky side chains reduced potencies and efficacies on both receptors (**3.28**, **3.40**, **3.50**, **3.67**, **3.68**, **3.76**, **3.77**, **3.82**, **3.83** and **3.85**). Introduction of a *para* hydroxy group at the aromatic ring of the 3-phenylpropanoyl analogs (**3.24** $\rightarrow$ **3.70**; **3.80** $\rightarrow$ **3.42**) resulted in decreased potencies but increased efficacies at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$. Compounds **3.42** and **3.70** were nearly full agonists at hH_2R-$Gs\alpha_S$ (E_{max} > 0.86) and gpH_2R-$Gs\alpha_S$ (E_{max} > 0.89), respectively. Notably, for compound **3.42** the preference for the recombinant gpH_2R relative to the hH_2R diminished significantly. The 3-methyl-4-phenylbutanoyl compounds (**3.72**, **3.44**) exhibited nearly the same efficacies as the 4-phenylbutanoyl derivatives (**3.26**, **3.37**) but had significantly lower potencies. The introduction of *meta* or *para* methoxy substituents, respectively, was well tolerated, whereas the *meta* substitution was slightly favored (**3.73** *vs.* **3.74**, **3.45** *vs.* **3.46**). Moreover, the branched 2-cyclohexyl-2-phenylacetyl residue in compound **3.38** caused a complete loss of agonistic activity at the hH_2R-$Gs\alpha_S$. Thus, hydrophobic properties of the acyl residue as well as sterical factors proved to play an important role in ligand - H_2R interaction.

Concerning guanidines bearing simple N^G-alkanoyl substituents, compounds **3.20** and **3.33** with a pentanoyl residue showed moderate H_2R agonistic activity at both hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins. Shortening (**3.19**, **3.32**) and elongation (**3.21**, **3.34**, **3.35**) of the carbon chain resulted in considerably reduced potencies or a complete loss of agonistic activity at the hH_2R-$Gs\alpha_S$ (**3.35**). Introduction of a thiol group in the side chain (**3.56**) resulted in decreased potency, whereas the maximal reponse at the gpH_2R-$Gs\alpha_S$ was drastically increased. Compound **3.55**, the acetic acid thioester of **3.56**, was 7-fold more potent at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$. Most notably, the 11-aminoundecanoyl guanidines **3.31** and **3.54** were potent H_2R agonists with up to 45 and 230 times higher potencies than histamine at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Comparison of the agonistic potency of **3.54** with the 6-aminohexanoyl guanidine **3.53** and the alkanoyl

guanidines **3.32-3.35** suggested that the increase in potency resulted from the additional basic group at appropriate distance to the pharmacophore and that the contribution of the hydrophobic alkyl linker was rather low (cf. Figure 3.4). Notably, masking of the basic amino group in **3.31** by propionylation (**3.59**) resulted in a decrease in potency by a factor of 2-3 at both receptors, whereas efficacies were not affected.

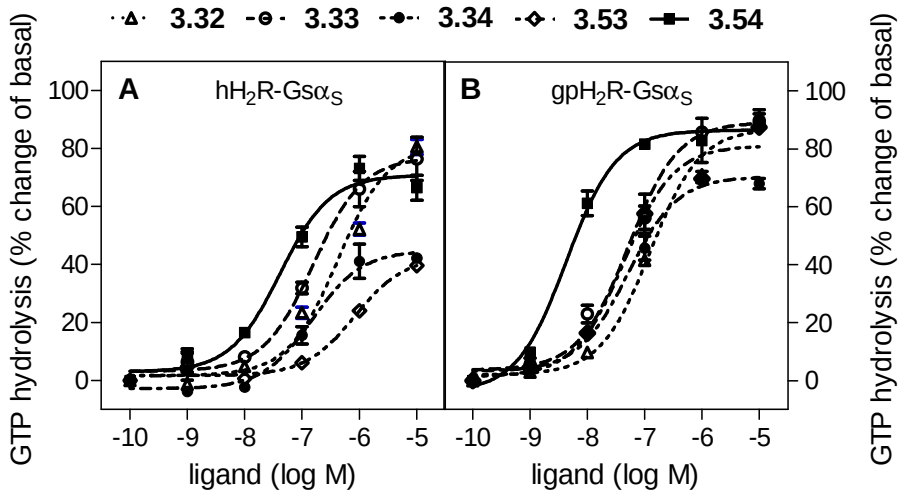


Figure 3.2. Effects of **3.32-3.34**, **3.53** and **3.54** on the GTPase activity. Mean values ± SEM of representative experiments performed in duplicate in membranes expressing hH_2R-$Gs\alpha_S$ (**A**) and gpH_2R-$Gs\alpha_S$ (**B**). Data are expressed as percentage change in GTPase activity relative to the effect induced by histamine (100 µM) = 100 %.

In principle, a free amino group in the acyl residue of the molecules allows for convenient fluorescence and radio labeling. Recently, such an approach was developed in our workgroup for the labeling of argininamide-type neuropeptide Y (NPY) Y_1 receptor antagonists.[20] In those NPY receptor ligands, space-filling acyl moieties attached to the guanidine group were tolerated without drastic decrease in activity. Therefore, prototypical compounds were synthesized to explore the applicability of this labeling strategy to acylguanidine-type radiotracers and fluorescent ligands for the H_2R. The free amino groups in compounds **3.31**, **3.53** and **3.71** were acylated with the "cold" versions of succinimidyl propionate (**3.31** → **3.59**, **3.53** → **3.62**) or 4-F-benzoate (**3.53** → **3.63**, **3.71** → **3.57**) or the succinimidyl ester of the cyanine dye S0586 (**3.31** → **3.61**). In addition, **3.31** and **3.71** were derivatized with the fluorescent pyrylium dye py-1 (**3.31** → **3.60**, **3.71** → **3.58**).

Table 3.2. Potencies and efficacies of N^G-acylated aminothiazolylpropylguanidines at hH_2R-Gsα_S and gpH_2R-Gsα_S fusion proteins in the steady-state GTPase assay.[a]

Compd.	hH_2R-Gsα_S			gpH_2R-Gsα_S			EC_{50} (hH_2R-Gsα_S) / EC_{50} (gpH_2R-Gsα_S)
	pEC_{50} ± SEM	E_{max} ± SEM	Pot_{rel}	pEC_{50} ± SEM	E_{max} ± SEM	Pot_{rel}	
His[13]	5.90 ± 0.09	1.00	1.0	5.92 ± 0.09	1.00	1.0	1.05
Amt[13]	6.72 ± 0.10	0.91 ± 0.02	6.6	6.72 ± 0.09	1.04 ± 0.01	6.3	1.00
3.64	6.01 ± 0.05	0.32 ± 0.0	1.3	6.37 ± 0.07	0.76 ± 0.02	2.8	2.19
3.65	5.48 ± 0.02	0.34 ± 0.03	0.4	5.91 ± 0.11	0.69 ± 0.05	1.0	2.57
3.19	5.83 ± 0.27	0.62 ± 0.04	0.9	6.71 ± 0.02	0.91 ± 0.03	6.2	7.24
3.20	7.06 ± 0.03	0.69 ± 0.03	14.5	7.54 ± 0.01	0.82 ± 0.02	41.7	2.88
3.21	7.02 ± 0.15	0.52 ± 0.07	13.2	7.46 ± 0.29	0.69 ± 0.06	34.7	2.63
3.22	5.83 ± 0.04	0.56 ± 0.02	0.9	6.52 ± 0.14	0.80 ± 0.06	4.0	4.68
3.23	7.02 ± 0.03	0.68 ± 0.01	13.2	7.67 ± 0.33	0.79 ± 0.01	56.2	4.27
3.24	7.69 ± 0.13	0.77 ± 0.02	61.7	8.13 ± 0.05	0.76 ± 0.02	162.2	2.63
3.25	7.83 ± 0.10	0.66 ± 0.04	85.1	8.08 ± 0.20	0.80 ± 0.09	144.5	1.70
3.26	7.66 ± 0.16	0.63 ± 0.02	57.5	7.86 ± 0.06	0.68 ± 0.07	87.1	1.51
3.27	7.54 ± 0.14	0.49 ± 0.01	43.7	7.92 ± 0.24	0.46 ± 0.02	100.0	2.29
3.66	7.82 ± 0.17	0.75 ± 0.03	83.2	8.55 ± 0.07	0.76 ± 0.08	426.6	5.13
3.67	7.38 ± 0.20	0.49 ± 0.01	30.2	8.14 ± 0.11	0.68 ± 0.06	166.0	5.50
3.68	7.70 ± 0.07	0.52 ± 0.04	63.1	8.44 ± 0.09	0.85 ± 0.09	331.1	5.25
3.28	7.04 ± 0.07	0.45 ± 0.04	13.8	7.98 ± 0.18	0.84 ± 0.02	114.8	8.32
3.69	7.56 ± 0.24	0.75 ± 0.07	45.7	8.16 ± 0.23	0.73 ± 0.07	173.8	3.80
3.70	7.52 ± 0.03	0.86 ± 0.02	41.7	8.07 ± 0.24	0.88 ± 0.07	141.3	3.39
3.71	6.83 ± 0.04	0.66 ± 0.04	8.5	8.16 ± 0.32	1.03 ± 0.11	173.8	20.42
3.72	7.39 ± 0.02	0.63 ± 0.03	30.9	7.59 ± 0.32	0.73 ± 0.03	46.8	1.51
3.73	7.44 ± 0.19	0.69 ± 0.03	34.7	7.87 ± 0.17	0.66 ± 0.02	89.1	2.57
3.74	7.12 ± 0.07	0.48 ± 0.01	16.6	7.56 ± 0.19	0.50 ± 0.05	43.7	2.63
3.29	7.29 ± 0.10	0.72 ± 0.01	24.6	7.80 ± 0.22	0.78 ± 0.06	75.9	3.09
3.30	7.31 ± 0.04	0.71 ± 0.08	25.7	7.78 ± 0.23	0.83 ± 0.09	72.4	2.82
3.75	7.88 ± 0.16	0.62 ± 0.03	95.5	8.18 ± 0.23	0.54 ± 0.13	182.0	1.91
3.76	7.42 ± 0.03	0.20 ± 0.01	33.1	7.76 ± 0.16	0.52 ± 0.05	69.2	2.09
3.77	7.83 ± 0.01	0.64 ± 0.02	85.1	8.10 ± 0.11	0.82 ± 0.02	151.4	1.78
3.78	7.61 ± 0.03	0.42 ± 0.03	51.3	7.85 ± 0.16	0.62 ± 0.04	45.7	0.89
3.79	7.99 ± 0.13	0.19 ± 0.02	123.0	8.37 ± 0.10	0.41 ± 0.01	281.8	2.29
3.31	7.45 ± 0.14	0.66 ± 0.07	35.5	8.00 ± 0.09	0.86 ± 0.03	120.2	3.39
3.57	6.51 ± 0.17	0.47 ± 0.01	4.1	6.60 ± 0.10	0.57 ± 0.01	4.8	1.17
3.58	6.27 ± 0.09	0.42 ± 0.07	2.4	6.53 ± 0.03	0.82 ± 0.02	4.1	1.74
3.59	7.09 ± 0.01	0.63 ± 0.04	15.5	7.58 ± 0.10	0.91 ± 0.06	45.7	2.95

Table 3.2. (continued)

3.60	6.95	0.49	11.2	7.19 ± 0.07	0.52 ± 0.07	18.6	1.66
3.61	5.52	0.46	0.4	5.46	0.32	0.4	0.83
3.32	6.44 ± 0.06	0.76 ± 0.04	3.5	6.90	0.85	9.6	2.75
3.33	6.82 ± 0.02	0.73 ± 0.01	8.3	7.28 ± 0.01	0.82 ± 0.04	22.9	2.75
3.34	7.11 ± 0.06	0.59 ± 0.01	16.2	7.36 ± 0.05	0.83 ± 0.02	27.5	1.70
3.35	(-	-	-)[b]	5.43 ± 0.17	0.39 ± 0.07	0.3	-
3.80	7.63 ± 0.03	0.82 ± 0.02	53.7	8.01 ± 0.15	0.80 ± 0.18	123.0	2.29
3.36	7.17 ± 0.05	0.75 ± 0.01	18.6	7.50 ± 0.03	0.92 ± 0.01	38.0	2.04
3.37	7.25 ± 0.03	0.66 ± 0.02	22.4	7.55 ± 0.0	0.82 ± 0.01	42.7	1.91
3.38	(-	-	-)[b]	6.41 ± 0.02	0.33 ± 0.01	3.1	-
3.39	7.41 ± 0.04	0.77 ± 0.02	32.4	7.87 ± 0.02	0.91 ± 0.02	89.1	2.75
3.40	7.12 ± 0.0	0.51 ± 0.01	16.6	7.31 ± 0.03	0.74 ± 0.05	24.6	1.48
3.81	7.57 ± 0.13	0.81 ± 0.07	46.8	8.21 ± 0.15	0.84 ± 0.04	195.0	4.17
3.82	7.53 ± 0.09	0.67 ± 0.05	42.7	7.69 ± 0.20	0.87 ± 0.21	58.9	1.38
3.83	7.33 ± 0.17	0.66 ± 0.05	26.9	8.15 ± 0.24	0.88 ± 0.06	169.8	6.31
3.41	7.38 ± 0.18	0.74 ± 0.02	30.2	7.76 ± 0.21	1.00 ± 0.03	69.2	2.29
3.42	7.46 ± 0.03	0.90 ± 0.04	36.3	7.45 ± 0.04	0.93 ± 0.02	33.9	0.93
3.43	6.57 ± 0.07	0.52 ± 0.05	4.7	7.71 ± 0.11	0.91 ± 0.02	61.7	13.80
3.44	7.10 ± 0.10	0.70 ± 0.03	15.9	7.23 ± 0.02	0.93 ± 0.01	20.4	1.29
3.45	7.16 ± 0.06	0.69 ± 0.01	18.2	7.27 ± 0.07	0.87 ± 0.02	22.4	1.23
3.46	7.09 ± 0.05	0.49 ± 0.02	15.5	7.13 ± 0.05	0.58 ± 0.02	16.2	1.05
3.47	7.23 ± 0.01	0.73 ± 0.04	21.4	7.47 ± 0.01	0.99 ± 0.04	35.5	1.66
3.48	7.65 ± 0.01	0.74 ± 0.01	56.2	8.09 ± 0.02	0.93 ± 0.01	147.9	2.63
3.49	7.28 ± 0.09	0.56 ± 0.02	24.0	7.71 ± 0.24	0.80 ± 0.01	61.7	2.57
3.84	7.70 ± 0.18	0.72 ± 0.07	63.1	7.97 ± 0.06	0.79 ± 0.11	112.2	1.78
3.85	7.90 ± 0.13	0.68 ± 0.05	100.0	8.22 ± 0.33	0.88 ± 0.06	199.5	2.00
3.50	7.47 ± 0.07	0.17 ± 0.01	37.2	7.31 ± 0.21	0.61 ± 0.02	24.6	0.66
3.51	7.36 ± 0.03	0.49 ± 0.0	28.8	7.43 ± 0.22	0.72 ± 0.02	32.4	1.12
3.52	7.26 ± 0.0	0.26 ± 0.02	22.9	7.36 ± 0.04	0.45 ± 0.01	27.5	1.20
3.53	6.36 ± 0.28	0.42 ± 0.01	2.9	7.35 ± 0.05	0.79 ± 0.04	26.9	9.33
3.54	7.55 ± 0.17	0.69 ± 0.01	44.7	8.29 ± 0.07	0.92 ± 0.02	234.4	5.25
3.55	7.48 ± 0.13	0.75 ± 0.03	38.0	7.74 ± 0.12	0.85 ± 0.01	66.1	1.74
3.56	6.64 ± 0.02	0.54 ± 0.11	5.5	6.86 ± 0.11	1.04 ± 0.05	8.7	1.58
3.62	6.25 ± 0.06	0.74 ± 0.01	2.2	6.38 ± 0.01	0.76 ± 0.0	2.9	1.29
3.63	7.00 ± 0.01	0.75 ± 0.02	12.6	7.36 ± 0.08	0.58 ± 0.07	27.5	2.29

[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R-Gsα_S and gpH_2R-Gsα_S was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 10 μM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 2.5 $pmol \cdot mg^{-1} \cdot min^{-1}$, and activities stimulated by histamine (100 μM) ranged between ≈ 2 and 13 $pmol \cdot mg^{-1} \cdot min^{-1}$. The efficacy ($E_{max}$) of histamine was determined by nonlinear

regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of 1-4 independent experiments, each performed in duplicate. The relative potency of histamine was set to 1.0, and the potencies of other agonists were referred to this value. [b] No agonistic activity.

Masking of the free amino groups with propionate or 4-F-benzoate, respectively, resulted in considerably decreased potencies (except for **3.63**) at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$. Moreover, **3.61**, coupled to the cyanine dye (S0586), showed only negligible activities at both recombinant H_2Rs ($pEC_{50} \leq 5.5$), whereas the py-labeled compounds **3.58** and **3.60** retained weak to moderate H_2R agonistic activities ($pEC_{50} \leq 7.2$). Fluorescent H_2R agonists should be useful pharmacological tools for studies on the cellular level, for example, to investigate receptor internalization. Unfortunately, the new fluorescence-labeled compounds turned out to be inappropriate for confocal microscopy due to low specific binding (**3.61**) or receptor-independent diffusion through the cell membrane (**3.58**, **3.60**). Thus, there is no universal recipe for labeling of GPCR ligands. The optimization of the structures for individual biological targets with respect to potency and physicochemical properties is indispensable.

In accordance with previous results for alkylated and acylated imidazolylalkylguanidines, the aminothiazolylpropylguanidines described in this chapter exhibited higher potencies and efficacies at gpH_2R-$Gs\alpha_S$ compared to hH_2R-$Gs\alpha_S$.[3-4,13,21] In particular, a free amino group (**3.43**, **3.53**, **3.54** and **3.71**) enhanced the preference for the gpH_2R ortholog. Figure 3.3 shows the comparison of potencies and efficacies of selected N^G-acylated aminothiazolylpropylguanidines at hH_2R-$Gs\alpha_S$ versus gpH_2R-$Gs\alpha_S$. Very recently, the highest ratio of EC_{50} values (EC_{50} (hH_2R-$Gs\alpha_S$)/ EC_{50} (gpH_2R-$Gs\alpha_S$)) was found for compound **3.71** with a 6-amino-3-phenylhexanoyl residue.[4] The high species-dependent preference was confirmed for the corresponding unmethylated analog **3.43**. These compounds exhibited moderate agonistic activities at hH_2R-$Gs\alpha_S$, but were 14-20 times more potent at gpH_2R-$Gs\alpha_S$ and therefore exhibited the highest selectivity towards gpH_2R-$Gs\alpha_S$ within this series of H_2R agonists. Notably, derivatization of the free amino group diminished the preference for the gpH_2R species ortholog, for example, **3.71** versus **3.57** (ratio of EC_{50} values: 20.4 → 1.17) and **3.71** versus **3.58** (20.4 → 1.74).

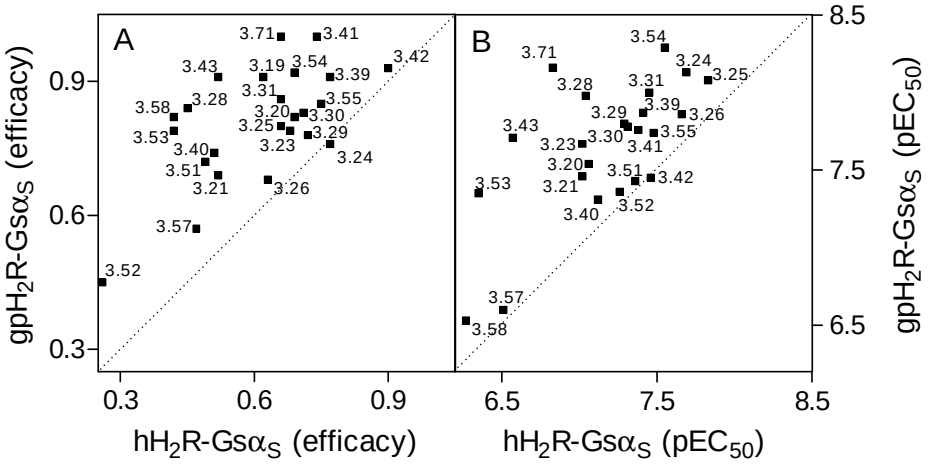


Figure 3.3. Efficacies and potencies of selected title compounds (**3.19-3.21**, **3.23-3.26**, **3.28-3.31**, **3.39-3.43**, **3.51-3.55**, **3.57**, **3.58** and **3.71**) at hH_2R-$Gs\alpha_S$ in comparison with gpH_2R-$Gs\alpha_S$ as determined in the steady-state GTPase assay. The dotted lines represent the line of identity. **A:** Plot of efficacies at gpH_2R-$Gs\alpha_S$ *vs.* hH_2R-$Gs\alpha_S$. **B:** Plot of pEC_{50} at gpH_2R-$Gs\alpha_S$ *vs.* hH_2R-$Gs\alpha_S$.

3.3.1.2 H_2R agonism on the isolated guinea pig right atrium

In addition to the GTPase assay, selected compounds were investigated on the isolated spontaneously beating guinea pig right atrium as a more complex, well established standard model for the characterization of H_2R ligands. As reported recently,[4] compared with the gpH_2R-$Gs\alpha_S$ fusion protein the potencies of the aminothiazolylpropylguanidines were lower at the gp right atrium (Table 3.3), but the order of potency was essentially in good agreement. The most potent H_2R agonists surpassed the potency of histamine by a factor of about 40. Aminothiazoles lacking the 4-methyl substituent showed slightly higher potencies relative to their methylated analogs, for example, **3.24** versus **3.80** and **3.77** versus **3.85**. The positive chronotropic response was mediated by the H_2R since it could be blocked by the H_2R antagonist cimetidine (10-100 µM). Typical competition experiments are shown for **3.71** in Figure 3.4.[4]

Table 3.3. H_2R agonism on the guinea pig right atrium.

Compd.	pEC_{50} ± SEM[a]	E_{max} ± SEM[b]	Pot_{rel}[c]
His	6.00 ± 0.02	1.0 ± 0.02	1.0
Amt[22]	6.21 ± 0.09	0.95 ± 0.02	1.6
3.23	6.22 ± 0.01	0.82 ± 0.03	1.7
3.24	6.72 ± 0.04	0.78 ± 0.02	5.8
3.71[4]	7.55 ± 0.03	0.97 ± 0.02	35.3
3.30	6.61 ± 0.07	0.86 ± 0.02	4.1
3.75[4]	6.75 ± 0.10	0.65 ± 0.03	5.7
3.77[4]	7.25 ± 0.12	0.70 ± 0.03	17.6
3.80[4]	7.18 ± 0.04	0.92 ± 0.02	15.2
3.83[4]	7.54 ± 0.08	0.74 ± 0.05	34.7
3.85[4]	7.61 ± 0.12	0.74 ± 0.04	40.7

[a] pEC_{50} values were calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$; summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, N = 16); the SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50} for 3-7 experiments. [b] Intrinsic activity, maximal response, relative to the maximal increase in heart rate induced by the reference compound histamine (30 µM) = 1.0. [c] Potency relative to histamine = 1.0.

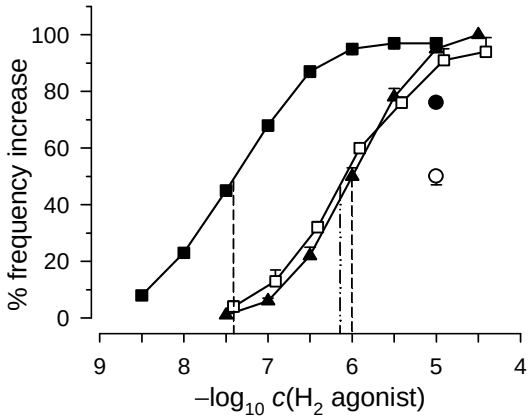


Figure 3.4. Concentration-response curves on the guinea pig right atrium. Histamine (▲, pEC_{50} = 6.00 ± 0.06, N = 4), **3.71** alone (■, pEC_{50} = 7.42 ± 0.04, relative potency 3,530 % (95 % confidence limits 2,900 – 4,310), E_{max} = 97 ± 2, N = 4) and **3.71** (□) in the presence of the H_2R antagonist cimetidine (10 µM, preincubation for 30 min, pA_2 = 6.24 ± 0.12, N = 2). Addition of cimetidine (30 µM and 100 µM, preincubation for 60 min each) led to a fading of the maximum response induced by **3.71** (10 µM, ■) to 76 ± 2 % (●) and 50 ± 4 % (○) (N = 4 each). Also from these experiments, affinity of cimetidine was estimated to be pA_2 = 6.32 ± 0.08 and 6.40 ± 0.05, respectively (N = 4 each) by measuring the horizontal distance of ● and ○ relative to the agonist curve (■).[4]

3.3.2 Receptor selectivity

To determine the histamine receptor selectivity profile (human H_2R *vs.* H_1R, H_3R, H_4R), representative compounds were investigated in GTPase assays on recombinant human H_1, H_3 and H_4 receptors for agonism and antagonism, respectively (Table 3.4). Except for compounds **3.31** and **3.54**, which also showed moderate antagonistic effects at the hH_1R, the investigated N^G-acylated aminothiazolylpropylguanidines showed no agonistic or relevant antagonistic effects in the GTPase assays on hH_1R, hH_3R and hH_4R. It can be speculated whether the moderate antagonistic effects at the hH_1R of the two 11-aminoundecanoyl guanidines (**3.31**, **3.54**) depend on the free amino group in the side chain, as capping of the amino group resulted in a drop of the antagonistic effect (**3.31** *vs.* **3.59**). However, all other 2-aminothiazoles containing free amino functions (**3.43**, **3.53** and **3.71**) showed only negligible effects at non-H_2 histamine receptors. Thus, in agreement with recent results the investigated N^G-acylated aminothiazolylpropyl-guanidines are highly selective for the H_2R.[2,4] These data confirm the working hypothesis that the 2-aminothiazole and the imidazole moiety are bioisosteric groups at the H_2R but not at the H_3R and the H_4R.[4]

Table 3.4. Agonistic, antagonistic and inverse agonistic effects of bivalent ligands at hH_1R + RGS4, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ + RGS4 and hH_4R-RGS19 + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ expressed in Sf9 cell membranes.[a]

Compd.	hH_1R	hH_3R	hH_4R	Compd.	hH_1R	hH_3R	hH_4R
	pK_B	pK_B	pK_B		pK_B	pK_B	pK_B
3.20	< 5.00	< 5.00	< 5.00	**3.53**	< 6.00	nd[b]	nd[b]
3.24	< 6.00	< 5.00	< 5.00	**3.54**	7.48 ± 0.01	nd[b]	nd[b]
3.28	< 6.00	< 5.00	< 6.00	**3.59**	< 6.00	< 6.00	< 6.00
3.31	7.06 ± 0.09	< 5.00	< 5.00	**3.71**	< 5.00	< 5.00	< 6.00
3.42	< 5.00	< 5.00	< 5.00	**3.78**	< 6.00	< 6.00	< 6.00
3.43	< 6.00	nd[b]	nd[b]	**3.80**	< 5.00	< 5.00	< 5.00
3.44	< 6.00	< 5.00	< 5.00	**3.81**	< 5.00	< 5.00	< 5.00
3.48	< 6.00	< 5.00	< 6.00	**3.83**	< 5.00	< 5.00	< 6.00
3.51	< 5.00	< 5.00	< 6.00				

[a] Steady state GTPase activity in Sf9 membranes expressing hH_1R+RGS4, hH_3R+$G\alpha_{i2}$+$G\beta_1\gamma_2$+RGS4 and hH_4R-RGS19+$G\alpha_{i2}$+$G\beta_1\gamma_2$ was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 100 µM as appropriate to generate saturated concentration-response curves. For the determination of antagonism, reaction mixtures contained histamine (hH_1R: 1 µM; hH_3R, hH_4R: 100 nM) and ligands at concentrations from 1 nM to 1 mM. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities stimulated by histamine (10 µM) ranged between ≈ 3.0 and 4.5 $pmol \cdot mg^{-1} \cdot min^{-1}$. Data

shown are mean values from one to three experiments performed in duplicate. IC_{50} values were converted to K_B values using the Cheng-Prusoff equation.[23] [b] nd: not determined.

3.4 Summary

Based on previous studies,[4,10] N^G-acylated aminothiazolylpropylguanidines were synthesized and pharmacologically characterized to gain more insight into the structure-activity relationships and to develop selective H_2R agonists as pharmacological tools for more detailed investigations of the biological role of the H_2R. The title compounds proved to be partial to full agonists at the guinea pig right atrium as well as at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins, respectively. The replacement of a phenyl with a cyclohexyl ring resulted mainly in higher potencies and efficacies at both H_2R orthologs. Highest potency resided in compounds having a two- to three-membered carbon chain between carbonyl group and phenyl or cyclohexyl ring, respectively. Whereas methyl substituents in α- or β-position to the carbonyl group in 3-phenyl- and 3-cylohexyl-propanoyl compounds were well tolerated, the introduction of more bulky side chains reduced the potency and efficacy at the H_2Rs. Notably, the introduction of a free amino group at an appropriate distance to the pharmacophore was beneficial with respect to H_2R agonistic potency. The H_2R agonistic activities of analogs lacking the 4-methyl group at the thiazole ring indicate that, in contrast to amthamine, this methyl substituent neither increased the agonistic activity for acylguanidine-type compounds in the GTPase assay nor at the gp right atrium. Moreover, in accordance to the structure-activity relationships of N^G-acylated imidazolylpropylguanidines, all investigated aminothiazolylpropyl-guanidines exhibited higher potencies and efficacies at gpH_2R-$Gs\alpha_S$ compared to hH_2R-$Gs\alpha_S$. In particular, a free amino group (**3.43**, **3.53**, **3.54** and **3.71**) enhanced the preference for the gpH_2R. Furthermore, investigation of the receptor selectivity profile (human H_2R *vs.* H_1R, H_3R, H_4R) revealed that N^G-acylated aminothiazolylpropyl-guanidines are highly selective for the H_2R. Whereas compounds of the imidazole series are very potent agonists or antagonists at the hH_3R and hH_4R, respectively, the investigated N^G-acylated aminothiazolylpropylguanidines showed only negligible effects at non-H_2 histamine receptors. Thus, this study substantiates previous results, confirming that the 2-aminothiazole and the imidazole moiety are bioisosteric groups at the H_2R but not at the H_3R and H_4R.

3.5 Experimental section

3.5.1 Chemistry

3.5.1.1 General conditions

Commercially available reagents were purchased from Acros Organics (Geel, Belgium), Lancaster Synthesis GmbH (Frankfurt, Germany), Sigma-Aldrich Chemie GmbH (München, Germany), Alfa Aesar GmbH & Co KG (Karlsruhe, Germany), Iris Biotech GmbH (Marktredwitz, Germany) or Merck KGaA (Darmstadt, Germany) and used as received. Where indicated, reactions were carried out under a dry, oxygen-free argon atmosphere. All solvents used were of analytical grade or distilled before use. THF and Et_2O were distilled over Na, DCM was predried over $CaCl_2$ or distilled from P_2O_5 and stored under argon atmosphere over molecular sieves 3 Å. Column chromatography was carried out using Merck silica gel Geduran 60 (0.063-0.200) and Merck silica gel 60 (0.040-0.063) for flash column chromatography. In certain cases, flash chromatography was performed on an Intelli Flash 310 Flash Chromatography Workstation from Varian Deutschland GmbH (Darmstadt, Germany). Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminium sheets and spots were visualized with UV light at 254 nm.

Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on a Bruker Avance 300 spectrometer using per-deuterated solvents. The chemical shift δ is given in parts per million (ppm) with reference to the chemical shift of the residual protic solvent compared to tetramethylsilane (δ = 0 ppm). Multiplicities were specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet) as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95, a Finnigan SSQ 710A and on a Finnigan ThermoQuest TSQ 7000 spectrometer. Melting points (mp) were measured on a BÜCHI 530 electrically heated copper block apparatus using an open capillary and are uncorrected.

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was either a Eurosphere-100 (250 x 32 mm) (Knauer) or a Nucleodur-100 C_{18}ec (250 x 21 mm) (Macherey-Nagel, Düren, Germany), which were attached to the UV-detector model K-2000 (Knauer). UV-detection was done at 210 nm. The temperature was 25 °C and the flow rate 37 ml/min (Eurosphere-100) or 20 ml/min (Nucleodur-100 C_{18}ec), respectively. The mobile phase was 0.1% TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products equipped with an SN400 controller, P4000 pump, an AS3000 autosampler, and a Spectra Focus UV/Vis detector. Stationary phase was either a Eurosphere-100 C_{18} (250 x 4.0, 5 µM) column (Knauer, column A) or a Nucleodur-C_{18}HTec (250 x 4.0, 5µM) column (Macherey-Nagel, column B), thermostated at 30°C. As mobile phase, gradients of MeCN/TFA (0.05 % aq) were used. *Column A*: gradient mode: 0 min: MeCN/TFA (0.05% aq) 10:90, 20 min: 60:40, 23 min: 95:5, -33 min: 95:5; flow rate = 0.7 mL min^{-1}; t_0 = 3.318 min. *Column B*: gradient mode: 0 min: MeCN/TFA (0.05% aq) 10:90, 20 min: 60:40, 21 min: 95:5, -29 min: 95:5 ; flow rate = 0.75 mL min^{-1}; t_0 = 2.675 min; k`= ($t_R$– $t_0$)/$t_0$. Absorbance was detected at 210 nm. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. An overview of HPLC conditions, retention times ($t_R$), capacity factors (k`) and purities of the synthesized compounds is given in chapter 8.

3.5.1.2 Preparation of the guanidinylating reagent 3.3

S-Methylthiouronium iodide (3.1)[24]

Thiourea (9.2 g, 120 mmol) and methyl iodide (17 g, 120 mmol) in MeOH (100 ml) were refluxed for 1 h. After evaporation, the crude product was taken up in Et_2O, sucked off and washed twice with Et_2O to yield **3.1** (25.6 g, 117 mmol, 98 %) as white solid. The crude product was used in the next step without further purification. ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.88 (br s, 4H, N***H***$_2$), 2.57 (s, 3H, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 91 (M^+, 100); $C_2H_7IN_2S$ (218.06).

***N-tert*-Butoxycarbonyl-S-methylisothiourea (3.2)**[10]

To a solution of **3.1** (25.6 g, 117 mmol) in DCM/abs (200 ml) were added NEt_3 (11.8 ml, 117 mmol) and Boc_2O (25.6 g, 117 mmol) in DCM/abs (50 ml) and stirred for 24 h at room temperature. The mixture was subsequently washed with water and brine, and the organic phase was dried over $MgSO_4$. After removing the solvent under reduced pressure,

the crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) yielding **3.2** (14.5 g, 65 %) as white solid. ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.54 (br s, 2H, N***H***$_2$), 2.31 (s, 3H, C***H***$_3$), 1.40 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 191 (MH^+, 100); $C_7H_{14}N_2O_2S$ (190.26).

_N_-Benzyloxycarbonyl-_N`_-*tert*-butoxycarbonyl-S-methylisothiourea (3.3)[11]

To a solution of **3.2** (14.5 g, 76.5 mmol) in DCM/abs (150 ml) was added benzyl succinimidyl carbonate (CbzOSu, 19.1 g, 76.5 mmol) and stirred for 20 h at ambient temperature. The mixture was subsequently extracted with DCM and basified with Na_2CO_3 (pH 9-10). The organic phase was washed with water, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) yielding **3.3** (22.5 g, 91 %) as white solid. mp = 64 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 11.58 (br s, 1H, N***H***), 7.37 (m, 5H, Ar-***H***), 5.19 (s, 2H, C***H***$_2$-Ar), 2.40 (s, 3H, C***H***$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 325 (MH^+, 100); $C_{15}H_{20}N_2O_4S$ (324.40).

3.5.1.3 Preparation of N^G-Boc-protected building blocks 3.17 and 3.18

2-(5-Hydroxypentyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (3.4)[17]

5-Amino-1-pentanol (8.3 g, 80 mmol) and phthalic anhydride (11.9 g, 80 mmol) were heated to 80-100 °C for 3 h. After cooling, 40 ml ice cold water was added and extracted three times with $CHCl_3$. The organic phase was washed with 5 % $NaHCO_3$ and three times with H_2O and the organic phase was dried over $MgSO_4$. After removing of the solvent under reduced pressure, the crude product was subjected to flash chromatography (PE/EtOAc 70/30 v/v) yielding **3.4** (14 g, 75 %) as pale yellow solid. mp = 43 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.75 (m, 2H, Ar-***H***), 7.64 (m, 2H, Ar-***H***), 3.58 (m, 4H, Pht-C***H***$_2$, C***H***$_2$OH), 2.16 (s, 1H, O***H***), 1.63 (m, 2H, Pht-CH_2C***H***$_2$), 1.53 (m, 2H, C***H***$_2$$CH_2$OH), 1.34 (m, 2H, C***H***$_2$$CH_2$$CH_2$OH); CI-MS ($NH_3$) *m/z* (%): 251 ($M+NH_4^+$, 100); $C_{13}H_{15}NO_3$ (233.36).

2-(5-Oxohexyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (3.5)[17]

A mixture of phthalimide (8.2 g, 65 mmol), 6-chlorohexan-2-one (15.1 g, 112 mmol) and K_2CO_3 (10.4 g, 75 mmol) in 110 ml DMF was heated to 80 °C for 24 h. After cooling to room temperature, the mixture was added to ice cold water and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The

crude product was subjected to flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) yielding **3.5** (7.18 g, 79 %) as white solid. mp = 73-75 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.9-7.3 (m, 4H, Ar-***H***), 3.7 (m, 2H, COC***H***$_2$), 2.50 (m, 2H, C***H***$_2$-Pht), 2.15 (s, 3H, C***H***$_3$), 1.9-1.5 (m, 4H, $COCH_2C$***H***$_2$, $COCH_2CH_2C$***H***$_2$); CI-MS (NH_3) *m/z* (%): 263 ($M+NH_4^+$, 100), 246 (MH^+, 15); $C_{14}H_{15}NO_3$ (245.10).

5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentanal (3.6)[17]

Oxalyl chloride (3.2 ml, 37. 5 mmol) in 80 ml DCM/abs was cooled to -50 °C and DMSO (5.7 ml, 80 mmol) in 25 ml DCM/abs was added under stirring and argon atmosphere at such a rate that the temperature was maintained at -50 °C. After the addition was complete, stirring was continued for 15 min. A solution of **3.4** (7 g, 30 mmol) in 40 ml DCM/abs was added slowly and stirring was continued for another 15 min. After the addition of NEt_3 (22 ml, 160 mmol), the mixture was allowed to warm to room temperature, 80 ml H_2O was added and stirring continued for 30 min. The organic phase was separated and washed with H_2O to almost neutral reaction. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure yielding crude **3.6** (6.24 g, 90 %) as yellow oil which was stored under argon and used without further purification. ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.75 (t, ^3J = 1.5 Hz, 1H, CO***H***), 7.82 (m, 2H, Ar-***H***), 7.70 (m, 2H, Ar-***H***), 3.70 (t, ^3J = 6.8 Hz, 2H, Pht-C***H***$_2$), 2.50 (m, 2H, C***H***$_2$COH), 1.69 (m, 4H, Pht-CH_2C***H***$_2CH_2$, Pht-CH_2CH_2C***H***$_2$); CI-MS (NH_3) *m/z* (%): 249 ($M+NH_4^+$, 100); $C_{13}H_{13}NO_3$ (231.25).

General procedure for the bromination of 3.5 and 3.6

To a solution of **3.5** or **3.6** (1 eq) in dioxane and DCM/abs (1.5:1) bromine (1 eq) was slowly added in a way that the brown color always disappeared. After complete addition of bromine the mixture was allowed to stir for 1 h at room temperature. Subsequently, the mixture was washed two times with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was obtained as yellow oil and used in the next step without further purification.

2-(4-Bromo-5-oxohexyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (3.7)[17]

The title compound was prepared from **3.5** (12.9 g, 53 mmol) in 250 ml dioxane and 165 ml DCM/abs and bromine (2.72 ml, 53 mmol) according to the general procedure yielding **3.7** as yellow oil (16.8 g, 98 %). ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.9-7.7 (m, 4H, Ar-*H*), 4.37 (m, 1H, C***H***Br), 3.77 (m, 2H, C***H***$_2$-Pht), 2.37 (s, 3H, COC***H***$_3$), 2.2-1.6 (m, 4H,

COCHC***H***$_2$CH$_2$, COCHCH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 324 (MH^+, 100); $C_{14}H_{14}NBrO_3$ (323.61).

2-Bromo-5-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentanal (3.8)[10,17]

The title compound was prepared from **3.6** (5.77 g, 25 mmol) in 150 ml dioxane and 100 ml DCM/abs and bromine (1.28 ml, 25 mmol) according to the general procedure yielding **3.8** (7.86 g, 100 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.44 (d, ^3J = 2.3 Hz, 1H, CO***H***), 7.83 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 4.34 (m, 1H, C***H***Br), 3.73 (t, ^3J = 6.6 Hz, 2H, Pht-C***H***$_2$), 1.87 (m, 4H, Pht-CH$_2$C***H***$_2$CH$_2$, Pht-CH$_2$CH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 329 (MNH_4^+, 100); $C_{13}H_{12}NO_3Br$ (310.14).

General procedure for the synthesis of the 2-aminothiazoles 3.9 and 3.10[4]

To a stirred solution of crude **3.7** or **3.8** (1 eq) in DMF, a solution of thiourea (1 eq) in DMF was added and the mixture was heated to 100 °C for 3 h. After cooling and removing the solvent *in vacuo*, a mixture of EtOAc/MeOH (1:1 v/v) was added and stirred for 30 min. Subsequently, the precipitate was filtered off, washed with EtOAc and Et_2O and the solid dried *in vacuo*.

2-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-1,3-dihydro-2*H*-isoindol-1,3-dione (3.9)[17]

The title compound was prepared from crude **3.7** (18.1 g, 56 mmol) in 50 ml DMF and a solution of thiourea (4.26 g, 56 mmol) in 50 ml DMF according to the general procedure yielding **3.9** (12 g, 71 %) as colorless solid. mp = 242 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.96 (s, 2H, N***H***$_2$), 7.84 (m, 4H, Ar-***H***), 3.62 (m, 2H, C***H***$_2$-Pht), 2.72 (m, 2H, Thiaz-5-C***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 302 (MH^+, 100); $C_{15}H_{15}N_3O_2S$ (301.4).

2-[3-(2-Aminothiazol-5-yl)propyl]-1,3-dihydro-2*H*-isoindol-1,3-dione (3.10)[4]

The title compound was prepared from crude **3.8** (7.86 g, 25.3 mmol) in 20 ml DMF and a solution of thiourea (1.9 g, 25.3 mmol) in 20 ml DMF according to the general procedure yielding **3.10** (14.71 g, 64 %) as light brown solid. ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.84 (m, 4H, Ar-***H***), 7.20 (s, 1H, Thiaz-4-***H***), 3.62 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$-Pht), 2.79 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 288 (MH^+, 100); $C_{14}H_{13}N_3O_2S$ (287.34).

General procedure for the *tert*-butoxycarbonyl protection of the 2-aminothiazoles 3.9 and 3.10 (3.11, 3.12)

Compound **3.9** and **3.10** (1 eq), respectively, was dissolved in $CHCl_3$ and Boc_2O (1.1 eq), NEt_3 (1.2) and DMAP (cat.) were added. The mixture was stirred overnight at ambient temperature. The mixture was extracted with DCM, the organic phase washed with 0.1N HCl, brine and water, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography.

***tert*-Butyl 4-methyl-5-[3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]thiazol-2-yl-carbamate (3.11)**[4]

The title compound was prepared from **3.9** (11.9 g, 40 mmol) in 100 ml $CHCl_3$, Boc_2O (9.6 g, 44 mmol), NEt_3 (6.7 ml, 48 mmol) and DMAP (cat.) according to the general procedure (PE/EtOAc 80/20 v/v) to obtain **3.11** (7.4 g, 46 %) as colorless foam-like solid. mp = 70-72 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.77 (m, 4H, Ar-***H***), 3.75 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$-Pht), 2.72 (t, ^3J = 7.8 Hz, 2H, Thiaz-5-C***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.98 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$); EI-MS (70 eV) *m/z* (%): 402 (MH^+, 100); $C_{20}H_{23}N_3O_4S$ (401.5).

***tert*-Butyl 5-[3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]thiazol-2-ylcarbamate (3.12)**[4]

Prepared from **3.10** (23 g, 80 mmol) in 200 ml $CHCl_3$, Boc_2O (19.2 g, 88 mmol), NEt_3 (13.3 ml, 96 mmol) and DMAP (cat.) according to the general procedure (PE/EtOAc 60/40 v/v) to obtain **3.12** (15.8 g, 51 %) as colorless foam-like solid. mp = 166 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.83 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 7.06 (s, 1H, Thiaz-4-***H***), 3.76 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$-Pht), 2.79 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.04 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.57 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 388 (MH^+, 100); $C_{19}H_{21}N_3O_4S$ (387.45).

General procedure for the preparation of the thiazolylpropylamines (3.13, 3.14) by hydrazinolysis of the phthalimides

To a suspension of **3.11** or **3.12** (1 eq) in EtOH was added hydrazine-monohydrate (5 eq). After stirring for 30 min at room temperature, the solution became clear and stirring was continued overnight. The mixture was cooled in an ice bath, the precipitate was removed by filtration and the filtrate evaporated to dryness. The crude product was subjected to flash chromatography ($CHCl_3$/MeOH/ NEt_3 94/5/1 v/v/v).

***tert*-Butyl 5-(3-aminopropyl)-4-methylthiazol-2-ylcarbamate (3.13)**[4]

The title compound was prepared from **3.11** (7.38 g, 18.4 mmol) in 70 ml EtOH and hydrazine-monohydrate (4.5 ml, 92 mmol) according to the general procedure yielding **3.13** (4.9 g, 98 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.70 (m, 4H, C***H***$_2$NH$_2$, Thiaz-5-C***H***$_2$), 2.23 (s, 3H, Thiaz-4-C***H***$_3$), 1.74 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 272 (MH^+, 100); $C_{12}H_{21}N_3O_2S$ (271.4).

***tert*-Butyl 5-(3-aminopropyl)thiazol-2-ylcarbamate (3.14)**[4]

The title compound was prepared from **3.12** (17.2 g, 44.5 mmol) in 170 ml EtOH and hydrazine-monohydrate (10.8 ml, 223.3 mmol) according to the general procedure yielding **3.14** (7.07 g, 62 %) as pale yellow solid. mp = 109 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.02 (s, 1H, Thiaz-4-***H***), 2.77 (m, 4H, C***H***$_2$NH$_2$, Thiaz-5-C***H***$_2$), 1.78 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 258 (MH^+, 100); $C_{11}H_{19}N_3O_2S$ (257.35).

General procedure for the guanidinylation of 3.13 and 3.14 with 3.3

NEt_3 (3 eq) was added to a suspension of the thiazolylpropylamines **3.13** or **3.14** (1 eq), **3.3** (1 eq) and $HgCl_2$ (2 eq) in DCM/abs and the mixture was stirred at ambient temperature for 48 h. Subsequently, EtOAc was added and the precipitate filtered over Celite. The crude product was purified by flash chromatography (PE/EtOAc 80/20 v/v).

***tert*-Butyl 5-[3-(3-benzyloxycarbonyl-2-*tert*-butyloxycarbonylguanidino)propyl]-4-methylthiazol-2-ylcarbamate (3.15)**[8]

The title compound was prepared from **3.13** (4.9 g, 18 mmol), **3.3** (5.84 g, 18 mmol), $HgCl_2$ (9.8 g, 36 mmol) and NEt_3 (7.5 ml, 54 mmol) in 500 ml DCM/abs and 500 ml EtOAc according to the general procedure yielding **3.15** (8.6 g, 87 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 11.4 (br s, 1H, N***H***), 9.6 (s, 1H, N***H***), 8.5 (t, ^3J = 5.1 Hz, 1H, CH$_2$N***H***), 7.34 (m, 5H, Ar-***H***), 5.14 (s, 2H, C***H***$_2$-Ar), 3.47 (m, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.48 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 548 (MH^+, 100); $C_{26}H_{37}N_5O_6S$ (547.67).

***tert*-Butyl 5-[3-(3-benzyloxycarbonyl-2-*tert*-butyloxycarbonylguanidino)propyl]-thiazol-2-ylcarbamate (3.16)**[4]

The title compound was prepared from **3.14** (6 g, 23.2 mmol), **3.3** (7.5 g, 23.2 mmol), $HgCl_2$ (12.6 g, 46.4 mmol) and NEt_3 (9.6 ml, 69.6 mmol) in 500 ml DCM/abs and 500 ml EtOAc according to the general procedure yielding **3.16** (11.76 g, 95 %) as colorless foam-like solid. mp = 140-142 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 11.35 (s, 1H, N***H***), 8.47 (t, ^3J = 5.4 Hz, 1H, CH_2N***H***), 7.34 (m, 5H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 5.13 (s, 2H, C***H***$_2$-Ph), 3.47 (m, 2H, C***H***$_2$NH), 2.75 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 534 (MH^+, 100); $C_{25}H_{35}N_5O_6S$ (533.64).

General procedure for the hydrogenolytic cleavage of Cbz groups (3.17, 3.18)[11]

To a solution of **3.15** or **3.16** in a mixture of THF/MeOH (1:1) was added Pd/C (10 %) and hydrogenated at 8 bar for 3-4 days (TLC control). The catalyst was removed by filtration over Celite and washed with MeOH. The solvent was removed *in vacuo*.

***tert*-Butyl 5-[3-(2-*tert*-butoxycarbonylguanidino)propyl]-4-methylthiazol-2-yl-carbamate (3.17)**

The title compound was prepared from **3.15** (8.54 g, 15.6 mmol) and 8 g of Pd/C (10 %) in a mixture of 160 ml THF/MeOH (1:1) according to the general procedure yielding **3.17** (4.38 g, 100 %) as white solid. mp = 111-114 °C; ^{1}H-NMR (CD_3OD) δ (ppm): 3.20 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.74 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.83 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.48 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 414 (MH^+, 100); $C_{18}H_{31}N_5O_4S$ (413.53).

***tert*-Butyl 5-[3-(2-*tert*-butoxycarbonylguanidino)propyl]thiazol-2-ylcarbamate (3.18)**

The title compound was prepared from **3.16** (5.8 g, 10.6 mmol) and 6 g of Pd/C (10 %) in a mixture of 160 ml THF/MeOH (1:1) according to the general procedure yielding **3.18** (3.39 g, 75 %) as colorless foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.03 (s, 1H, Thiaz-4-***H***), 3.26 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.80 (t, ^3J = 7.2 Hz, 2H, Thiaz-5-C***H***$_2$), 1.95 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.47 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 400 (MH^+, 100); $C_{17}H_{29}N_5O_4S$ (399.50).

3.5.1.4 Preparation of the Boc-protected N^G-acylated aminothiazolyl-propylguanidines 3.19a-3.55a

General procedure for the synthesis of 3.19a, 3.22a-3.32a and 3.35a-3.55a

DIEA (1 eq) was added to a solution of carboxylic acid (1 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs under argon and stirred for 15 min. A solution of **3.17** or **3.18** (1 eq) in DCM/abs was added and the mixture stirred overnight at room temperature. The solvent was removed under reduced pressure and EtOAc and water was added to the resulting residue. The organic phase was separated and the aqueous phase extracted twice with EtOAc. After drying over $MgSO_4$, the organic solvent was removed *in vacuo*. The crude product was purified by flash-chromatography (PE/EtOAc 80/20 v/v) unless otherwise indicated.

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-propionylguanidino]propyl}-4-methyl-thiazol-2-ylcarbamate (3.19a)

The title compound was prepared from propanoic acid (30 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.17** (165 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.19a** (170 mg, 91 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.46 (m, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.44 (q, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.17 (m, 3H, COCH$_2$C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 470.1 (MH^+, 100); $C_{21}H_{35}N_5O_5S$ (469.60).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-benzoylguanidino]propyl}-4-methyl-thiazol-2-ylcarbamate (3.22a)

The title compound was prepared from benzoic acid (61 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.22a** (200 mg, 78 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.17 (m, 2H, Ar-***H***), 7.58-7.35 (m, 3H, Ar-***H***), 3.60 (m, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.23 (s, 3H, Thiaz-4-C***H***$_3$), 2.01 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.51 (s, 18H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 518 (MH^+, 100); $C_{25}H_{35}N_5O_5S$ (517.24).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(2-phenylacetyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.23a)

The title compound was prepared from 2-phenylacetic acid (68 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3

ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.23a** (212.5 mg, 80 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35-7.20 (m, 5H, Ar-***H***), 3.67 (s, 2H, COC***H***$_2$), 3.43 (m, 2H, C***H***$_2$NH), 2.68 (t, ^3J = 7.1 Hz, 2H, Thiaz-5-C***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.46 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 532 (MH^+, 100); $C_{26}H_{37}N_5O_5S$ (531.25).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-*2*-(3-phenylpropanoyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.24a)**

The title compound was prepared from 3-phenylpropanoic acid (75 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.24a** (201.8 mg, 74 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30-7.15 (m, 5H, Ar-***H***), 3.45 (m, 2H, C***H***$_2$NH), 3.0 (m, 2H, C***H***$_2$-Ar), 2.78-2.65 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 546 (MH^+, 100); $C_{27}H_{39}N_5O_5S$ (545.27).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(4-phenylbutanoyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.25a)**

The title compound was prepared from 4-phenylbutanoic acid (82 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.25a** (223 mg, 80 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30-7.15 (m, 5H, Ar-***H***), 3.45 (m, 2H, C***H***$_2$NH), 2.62 (m, 4H, Thiaz-5-C***H***$_2$, C***H***$_2$-Ar), 2.40 (m, 2H, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.99 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.87 (m, 2H, COCH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 560 (MH^+, 100); $C_{28}H_{41}N_5O_5S$ (559.28).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(5-phenylpentanoyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.26a)**

The title compound was prepared from 5-phenylpentanoic acid (89 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.26a** (240.7 mg, 84 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ

(ppm): 7.30-7.10 (m, 5H, Ar-***H***), 3.40 (m, 2H, C***H***$_2$NH), 2.64 (m, 4H, Thiaz-5-C***H***$_2$, C***H***$_2$-Ar), 2.35 (m, 2H, COC***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.83 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.70-1.55 (m, 2H, COCH$_2$C***H***$_2$, COCH$_2$CH$_2$C***H***$_2$), 1.48 (s, 18H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 574.2 (MH$^+$, 100); $C_{29}H_{43}N_5O_5S$ (573.3).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(6-phenylhexanoyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.27a)

The title compound was prepared from 6-phenylhexanoic acid (96 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.27a** (240.8 mg, 82 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.18 (m, 5H, Ar-***H***), 3.46 (m, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.1 Hz, 2H, Thiaz-5-C***H***$_2$), 2.63 (m, 2H, C***H***$_2$-Ar), 2.38 (m, 2H, COC***H***$_2$), 2.19 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.76-1.57 (m, 6H, COCH$_2$C***H***$_2$, COCH$_2$CH$_2$C***H***$_2$, C***H***$_2$CH$_2$-Ar), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 588 (MH$^+$, 100); $C_{30}H_{45}N_5O_5S$ (587.31).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(3,3-diphenylpropanoyl)guanidino]-propyl}-4-methylthiazol-2-ylcarbamate (3.28a)

The title compound was prepared from 3,3-diphenylpropanoic acid (113 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.28a** (282.7 mg, 91 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.50-7.30 (m, 4H, Ar-***H***), 7.25-7.1 (m, 6H, Ar-***H***), 4.60 (m, 1H, C***H***(Ar)$_2$), 3.39 (m, 2H, C***H***$_2$NH), 3.12 (m, 2H, COC***H***$_2$), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.83 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.45 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 622 (MH$^+$, 100); $C_{33}H_{43}N_5O_5S$ (621.3).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(cyclohexanecarbonyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.29a)

The title compound was prepared from cyclohexanecarboxylic acid (64 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.29a** (220 mg, 84 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.46 (m, 2H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.30 (m, 1H,

COC***H***), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 2.0-1.76 (m, 10H, Thiaz-5-CH$_2$C***H***$_2$, cHex-***H***), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 524 (MH$^+$, 100); $C_{25}H_{41}N_5O_5S$ (523.28).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(2-cyclohexylacetyl)guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.30a)**

The title compound was prepared from 2-cyclohexylacetic acid (71 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.30a** (240 mg, 89 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.45 (m, 2H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.25 (m, 1H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.86-1.60 (m, 8H, cHex-***H***), 1.54 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 538 (MH$^+$, 100); $C_{26}H_{43}N_5O_5S$ (537.3).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-[11-(*tert*-butoxycarbonylamino)-undecanoyl]guanidino]propyl}-4-methylthiazol-2-ylcarbamate (3.31a)**

The title compound was prepared from 11-(*tert*-butoxycarbonylamino)undecanoic acid (226 mg, 0.75 mmol), EDAC (160 mg, 0.75 mmol), HOBt-monohydrate (127 mg, 0.75 mmol), DIEA (0.14 ml, 0.75 mmol) in 3 ml DCM/abs and **3.17** (310 mg, 0.75 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.31a** (300 mg, 57 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.45 (m, 2H, C***H***$_2$NH), 3.09 (m, 2H, C***H***$_2$NHBoc), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.39 (t, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$NH$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.44 (s, 9H, C(C***H***$_3$)$_3$), 1.28 (m, 12H, (C***H***$_2$)$_6$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 697.3 (MH$^+$, 100); $C_{34}H_{60}N_6O_7S$ (696.94).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-propanoylguanidino]propyl}thiazol-2-yl-carbamate (3.32a)**

The title compound was prepared from propanoic acid (30 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.32a** (150 mg, 82 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.04 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.79 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.44 (q, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 1.94 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.54 (s, 9H,

C(C***H***$_3$)$_3$), 1.48 (s, 9H, C(C***H***$_3$)$_3$), 1.17 (m, 3H, COCH$_2$C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 456.1 (MH$^+$, 100); $C_{20}H_{33}N_5O_5S$ (455.57).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-nonadecanoylguanidino]propyl}thiazol-2-ylcarbamate (3.35a)

The title compound was prepared from nonadecanoic acid (120 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-4 min: PE/EtOAc 100/0, 6-15 min: 80/20, 19-24 min: 50/50) yielded **3.32a** (150 mg, 82 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.04 (s, 1H, Thiaz-4-***H***), 3.47 (m, 2H, C***H***$_2$NH), 2.79 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.40 (m, 2H, COC***H***$_2$), 1.92 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.67 (m, 2H, COCH$_2$C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.31 (m, 2H, C***H***$_2$CH$_3$), 1.25 (m, 28H, (C***H***$_2$)$_{14}$), 0.88 (t, ^3J = 7.1 Hz, 3H, CH$_2$C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 680.6 (MH$^+$, 100); $C_{36}H_{65}N_5O_5S$ (680).

tert-Butyl 5-{3-[3(_tert_-butoxycarbonyl)-2-(4-phenylbutanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.36a)

The title compound was prepared from 4-phenylbutanoic acid (66 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.36a** (196 mg, 90 %) as yellow oil. ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 546.1 (MH$^+$, 100); $C_{27}H_{39}N_5O_5S$ (545.69).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(5-phenylpentanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.37a)

The title compound was prepared from 5-phenylvaleric acid (89 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.37a** (240 mg, 86 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.25-7.12 (m, 4H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.46 (m, 2H, C***H***$_2$NH), 2.82 (m, 2H, Thiaz-5-C***H***$_2$), 2.65 (m, 2H, C***H***$_2$Ar), 2.36 (m, 2H, COC***H***$_2$) 1.96 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.69 (m, 4H, COCH$_2$C***H***$_2$, C***H***2CH2Ar), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 560.3 (MH$^+$, 100); $C_{28}H_{41}N_5O_5S$ (559.72).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(2-cyclohexyl-2-phenylacetyl)-guanidino]propyl}thiazol-2-ylcarbamate (3.38a)**

The title compound was prepared from 2-cyclohexyl-2-phenylacetic acid (87 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.38a** (200 mg, 83 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.36-7.15 (m, 5H, Ar-***H***), 7.01 (s, 1H, Thiaz-4-C***H***), 3.50 (m, 1H, C***H***(Ar)cHex), 3.37 (t, ^{3}J = 6.9 Hz, 2H, C***H***$_2$NH), 2.79 (m, 2H, Thiaz-5-C***H***$_2$), 2.23 (m, 1H, cHex-***H***), 1.91 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.75-1.61 (m, 4H, cHex-***H***), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.46 (s, 9H, C(C***H***$_3$)$_3$), 1.27 (m, 6H, cHex-***H***); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 600.3 (MH^+, 100); $C_{31}H_{45}N_5O_5S$ (599.79).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(2-methyl-3-phenylpropanoyl)guanidino])-propyl}thiazol-2-ylcarbamate (3.39a)**

The title compound was prepared from 2-methyl-3-phenylpropanoic acid (82 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.39a** (240 mg, 88 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.25-7.12 (m, 5H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.45 (m, 2H, C***H***$_2$NH), 3.06 (m 1H, COC**H**), 2.79 (t. ^{3}J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.68 (m, 2H C***H***$_2$Ar), 1.93 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.09 (d, ^{3}J = 6.7 Hz, 3H, CHC***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 546.3 (MH^+, 100); $C_{27}H_{39}N_5O_5S$ (545.69).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(2-benzylbutanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.40a)**

The title compound was prepared from 2-benzylbutanoic acid (18 mg, 0.1 mmol), EDAC (20 mg, 0.1 mmol), HOBt-monohydrate (15 mg, 0.1 mmol), DIEA (17 µl, 0.1 mmol) in 3 ml DCM/abs and **3.18** (40 mg, 0.1 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-10 min: 80/20, -20 min: 50/50) yielded **3.40a** (40 mg, 67 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30-7.13 (m, 5H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.44 (m, 2H, C***H***$_2$NH), 3.02-2.74 (m, 4H, Thiaz-5-C***H***$_2$, C***H***$_2$Ar), 2.53 (m, 1H, COC**H**), 1.91 (m, 2H, Thiaz-5-CH_2C**H**$_2$), 1.70 (m, 2H, C***H***$_2$$CH_3$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.48 (s, 9H, C(C***H***$_3$)$_3$),

0.96 (t, ^3J = 7.41 Hz, 3H, $CH_2C\boldsymbol{H}_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 560.2 (MH^+, 100); $C_{28}H_{41}N_5O_5S$ (559.72).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(3-*p*-tolylbutanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.41a)**

The title compound was prepared from 3-*p*-tolylbutanoic acid (36 mg, 0.2 mmol), EDAC (39 mg, 0.2 mmol), HOBt-monohydrate (30 mg, 0.2 mmol), DIEA (34 µl, 0.2 mmol) in 3 ml DCM/abs and **3.18** (80 mg, 0.2 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-10 min: 80/20, -20 min: 50/50) yielded **3.41a** (75 mg, 67 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.11 (m, 4H, Ar-***H***), 7.03 (s, 1H, Thiaz-4-***H***), 3.43 (m, 2H, $C\boldsymbol{H}_2NH$), 3.28 (q, ^3J = 7.1 Hz, 1H, $C\boldsymbol{H}CH_3$), 2.76 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-$C\boldsymbol{H}_2$), 2.66-2.57 (m, 2H, $COC\mathbf{H}_2$), 2.31 (s, 3H, Ar-$C\boldsymbol{H}_3$), 1.90 (m, 2H, Thiaz-5-$CH_2C\mathbf{H}_2$), 1.56 (s, 9H, $C(C\boldsymbol{H}_3)_3$), 1.50 (s, 9H, $C(C\boldsymbol{H}_3)_3$), 1.47 (s, 3H, $CHC\boldsymbol{H}_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 560.2 (MH^+, 100); $C_{28}H_{41}N_5O_5S$ (559.72).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-[3(4-hydroxyphenyl)propanoyl]-guanidino]propyl}thiazol-2-ylcarbamate (3.42a)**

The title compound was prepared from 3-(4-hydroxyphenyl)propanoic acid (67 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.42a** (180 mg, 82 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.04 (m, 2H, Ar-***H***), 7.01 (s, 1H, Thiaz-4-***H***), 6.76(m, 2H, Ar-***H***), 3.46 (m, 2H, $C\boldsymbol{H}_2NH$), 2.92 (m, $C\boldsymbol{H}_2$-Ar), 2.76 (m, 2H, Thiaz-5-$C\boldsymbol{H}_2$), 2.68 (m, 2H, $COC\boldsymbol{H}_2$), 1.90 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.56 (s, 9H, $C(C\boldsymbol{H}_3)_3$), 1.48 (s, 9H, $C(C\boldsymbol{H}_3)_3$); $C_{26}H_{37}N_5O_6S$ (547.67).

***tert*-Butyl 5-(3-{3-(*tert*-butoxycarbonyl)-2-[6-(*tert*-butoxycarbonylamino)-3-phenylhexanoyl]guanidino}propyl)thiazol-2-ylcarbamate (3.43a)**

The title compound was prepared from 6-(*tert*-butoxycarbonylamino)-3-phenylhexanoic acid[10] (110 mg, 0.336 mmol), EDAC (69 mg, 0.36 mmol), HOBt-monohydrate (55 mg, 0.36 mmol), DIEA (62 µl, 0.36 mmol) in 3 ml DCM/abs and **3.18** (144 mg, 0.36 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.43a** (45 mg, 18 %) as white yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 345 ($(M+2H)^{2+}$, 100), 689.3 (MH^+, 75); $C_{34}H_{52}N_6O_7S$ (688.88).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(3-methyl-4-phenylbutanoyl)guanidino]-propyl}thiazol-2-ylcarbamate (3.44a)**

The title compound was prepared from 3-methyl-4-phenylbutanoic acid (45 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (43 µl, 0.25 mmol) in 3 ml DCM/abs and **3.18** (100 mg, 0.25 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-10 min: 80/20, -20 min: 50/50) yielded **3.44a** (110 mg, 79 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.32-7.15 (m, 5H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.46 (m, 2H, C***H***$_2$NH), 2.79 (t, ^{3}J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.64-2.16 (m, 5H, COC***H***$_2$, C***H***$_2$Ar, C***H***CH$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C**H**$_2$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.51 (s, 9H, C(C***H***$_3$)$_3$), 0.99 (d, ^{3}J = 6.6 Hz, 3H, CHC***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 560.2 (MH^+, 100); $C_{28}H_{41}N_5O_5S$ (559.72).

***tert*-Butyl 5-(3-{3-(*tert*-butoxycarbonyl)-2-[4-(3-methoxyphenyl)-3-methylbutanoyl]-guanidino}propyl)thiazol-2-ylcarbamate (3.45a)**

The title compound was prepared from 4-(3-methoxyphenyl)-3-methylbutanoic acid (79 mg, 0.38 mmol), EDAC (73 mg, 0.38 mmol), HOBt-monohydrate (58 mg, 0.38 mmol), DIEA (65 µl, 0.38 mmol) in 3 ml DCM/abs and **3.18** (152 mg, 0.38 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-10 min: 80/20, 12-18 min: 20/80) yielded **3.45a** (100 mg, 45 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.19 (m, 1H, Ar-***H***), 7.03 (s, 1H, Thiaz-4-***H***), 6.75 (m, 2H, Ar-***H***), 3.79 (s, 3H, OC***H***$_3$), 3.46 (m, 2H, C***H***$_2$NH), 2.78 (m, 2H, Thiaz-5-C***H***$_2$), 2.60-2.29 (m, 4H, COC***H***$_2$, Ar-C***H***$_2$), 2.18 (m, 1H, C***H***CH$_3$), 1.93 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 0.99 (d, ^{3}J = 6.31 Hz, 3H, CHC***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 590.3 (MH^+, 100); $C_{29}H_{43}N_5O_6S$ (589.75).

***tert*-Butyl 5-(3-{3-(*tert*-butoxycarbonyl)-2-[4-(4-methoxyphenyl)-3-methylbutanoyl]-guanidino}propyl)thiazol-2-ylcarbamate (3.46a)**

The title compound was prepared from 4-(4-methoxyphenyl)-3-methylbutanoic acid (65 mg, 0.31 mmol), EDAC (60 mg, 0.31 mmol), HOBt-monohydrate (47 mg, 0.31 mmol), DIEA (53 µl, 0.31 mmol) in 2 ml DCM/abs and **3.18** (124 mg, 0.31 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-12 min: 80/20, 13-20 min: 50/50) yielded **3.46a**

(80 mg, 44 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.07 (m, 2H, Ar-***H***), 7.03 (s, 1H, Thiaz-4-***H***), 6.81 (m, 2H, Ar-***H***), 3.77 (s, 3H, OC***H***$_3$), 3.46 (m, 2H, C***H***$_2$NH), 2.78 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.62-2.13 (m, 5H, COC***H***$_2$, Ar-C***H***$_2$, C***H***CH$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 0.97 (d, ^3J = 6.31 Hz, 3H, CHC***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 590.3 (MH^+, 100); $C_{29}H_{43}N_5O_6S$ (589.75).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(2-cyclohexylacetyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.47a)**

The title compound was prepared from 2-cyclohexylacetic acid (28 mg, 0.2 mmol), EDAC (39 mg, 0.2 mmol), HOBt-monohydrate (30 mg, 0.2 mmol), DIEA (34 µl, 0.2 mmol) in 3 ml DCM/abs and **3.18** (80 mg, 0.2 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-15 min: 80/20) yielded **3.47a** (70 mg, 67 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.08 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.80 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.27 (d, ^3J = 7.0 Hz, 2H, COC***H***$_2$) 1.93 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.73 (m, 7H, cHex-***H***), 1.55 (s, 9H, C(C***H***$_3$)$_3$), 1.51 (s, 9H, C(C***H***$_3$)$_3$), 1.25 (m, 4H, cHex-***H***); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 524.1 (MH^+, 100); $C_{25}H_{41}N_5O_5S$ (523.69).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(3-cyclohexylpropanoyl)guanidino]-propyl}thiazol-2-ylcarbamate (3.48a)**

The title compound was prepared from 3-cylcohexylpropanoic acid (78 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.48a** (240 mg, 89 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 538.3 (MH^+, 100); $C_{26}H_{43}N_5O_5S$ (537.72).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-(4-cyclohexylbutanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.49a)**

The title compound was prepared from 4-cyclohexylbutanoic acid (68 mg, 0.4 mmol), EDAC (77 mg, 0.4 mmol), HOBt-monohydrate (61 mg, 0.4 mmol), DIEA (69 µl, 0.4 mmol) in 3 ml DCM/abs and **3.18** (160 mg, 0.4 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.49a** (170 mg, 77 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.03 (s, 1H, Thiaz-4-***H***), 3.42 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.81 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.40 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.93 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$),

1.77-1.57 (m, 11H, $COCH_2C\boldsymbol{H}_2$, cHex-***H***), 1.53 (s, 9H, $C(C\boldsymbol{H}_3)_3$), 1.48 (s, 9H, $C(C\boldsymbol{H}_3)_3$), 1.29 (m, 2H, cHex-***H***), 1.24 (m, 2H, cHex-***H***); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 552.3 (MH^+, 100); $C_{27}H_{45}N_5O_5S$ (551.74).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(3-cyclohexyl-5-methylhexanoyl)-guanidino]propyl}thiazol-2-ylcarbamate (3.50a)

The title compound was prepared from 3-cyclohexyl-5-methylhexanoic acid (61 mg, 0.3 mmol), EDAC (58 mg, 0.3 mmol), HOBt-monohydrate (46 mg, 0.3 mmol), DIEA (52 µl, 0.3 mmol) in 3 ml DCM/abs and **3.18** (120 mg, 0.3 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.50a** (150 mg, 84 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 594.3 (MH^+, 100); $C_{30}H_{51}N_5O_5S$ (593.82).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(4-cyclohexyl-3-methylbutanoyl)-guanidino]propyl}thiazol-2-ylcarbamate (3.51a)

The title compound was prepared from 4-cyclohexyl-3-methylbutanoic acid (28 mg, 0.15 mmol), EDAC (30 mg, 0.15 mmol), HOBt-monohydrate (24 mg, 0.15 mmol), DIEA (27 µl, 0.15 mmol) in 3 ml DCM/abs and **3.18** (60 mg, 0.15 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.51a** (80 mg, 94 %) as yellow-brown oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 565.3 (MH^+, 100); $C_{28}H_{47}N_5O_5S$ (565.77).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(3-(cyclohexylmethyl)pentanoyl)-guanidino]propyl}thiazol-2-ylcarbamate (3.52a)

The title compound was prepared from 3-(cyclohexylmethyl)pentanoic acid (45 mg, 0.23 mmol), EDAC (44 mg, 0.23 mmol), HOBt-monohydrate (35 mg, 0.23 mmol), DIEA (39 µl, 0.23 mmol) in 3 ml DCM/abs and **3.18** (92 mg, 0.23 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.52a** (100 mg, 75 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580.3 (MH^+, 100); $C_{29}H_{49}N_5O_5S$ (579.79).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(6-(_tert_-butoxycarbonylamino)hexanoyl)-guanidino]propyl}thiazol-2-ylcarbamate (3.53a)

The title compound was prepared from 6-(*tert*-butoxycarbonylamino)hexanoic acid (230 mg, 1 mmol), EDAC (192 mg, 1 mmol), HOBt-monohydrate (153 mg, 1 mmol), DIEA (170 µl, 1 mmol) in 3 ml DCM/abs and **3.18** (400 mg, 1 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.53a** (490 mg, 82 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 613.2 (MH^+, 100); $C_{28}H_{48}N_6O_7S$ (612.78).

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-(11-aminoundecanoyl)guanidino]propyl}-thiazol-2-ylcarbamate (3.54a)

The title compound was prepared from 11-(*tert*-butoxycarbonylamino)undecanoic acid (250 mg, 0.83 mmol), EDAC (159 mg, 0.83 mmol), HOBt-monohydrate (127 mg, 0.83 mmol), DIEA (206 µl, 0.83 mmol) in 3 ml DCM/abs and **3.18** (330 mg, 0.83 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.54a** (420 mg, 74 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.03 (s, 1H, Thiaz-4-***H***), 3.48 m, 2H, C***H***$_2$NH), 3.09 (m, 2H, C***H***$_2$NHBoc), 2.79 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.39 (t, ^3J = 7.7 Hz, 2H, COC***H***$_2$), 1.92 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$NHBoc), 1.55 (s, 9H, (C***H***$_3$)$_3$), 1.50 (s, 9H, (CH$_3$)$_3$) 1.44 (s, 9H, (C***H***$_3$)$_3$), 1.28 (m, 12H, (C***H***$_2$)$_6$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 683.4 (MH^+, 100); $C_{33}H_{58}N_6O_7S$ (682.91).

S-6-((_tert_-Butoxycarbonylamino){3-[2-(_tert_-butoxycarbonylamino)thiazol-5-yl]-propylamino}methyleneamino)-6-oxohexyl ethanethioate (3.55a)

The title compound was prepared from 6-(acetylthio)hexanoic acid (95 mg, 0.5 mmol), EDAC (96 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (86 µl, 0.5 mmol) in 3 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **3.55a** (260 mg, 91 %) as dark yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.05 (s, 1H, Thiaz-4-***H***), 3.47 (m, 2H, C***H***$_2$NH), 2.86 (m, 2H, SC***H***$_2$), 2.79 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.40 (t, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.32 (s, 3H, COC***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.73-1.58 (m, 6H, SCH$_2$C***H***$_2$, COCH$_2$C***H***$_2$, COCH$_2$C***H***$_2$), 1.54 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 572.2 (MH^+, 100); $C_{25}H_{41}N_5O_6S_2$ (571.75).

General procedure for the synthesis of 3.20a, 3.21a, 3.33a and 3.34a

NEt_3 (1 eq) and the pertinent acid chloride (1 eq) was added to a solution of **3.17** or **3.18** (1 eq) in 4 ml DCM/abs. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted three times with EtOAc. After drying over $MgSO_4$, the solvent was removed *in vacuo*. The crude product was purified by flash chromatography.

tert-Butyl 5-{3-[3-(_tert_-butoxycarbonyl)-2-pentanoylguanidino]propyl}-4-methyl-thiazol-2-ylcarbamate (3.20a)

The title compound was prepared from **3.17** (165 mg, 0.4 mmol), NEt_3 (55 µl, 0.4 mmol) and pentanoyl chloride (47 µl, 0.4 mmol) in 4 ml DCM/abs according to the general procedure (PE/EtOAc 70/30 v/v) yielding **3.20a** (180 mg, 90 %) as pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.46 (m, 2H, C***H***$_2$NH), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.40 (m, 2H, COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.67 (m, 2H, $COCH_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.38 (m, 2H, C***H***$_2$$CH_3$), 0.96 (m, 3H, CH_2C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 498.1 (MH^+, 100); $C_{23}H_{39}N_5O_5S$ (497.65).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-nonanoylguanidino]propyl}-4-methyl-thiazol-2-ylcarbamate (3.21a)**

The title compound was prepared from **3.17** (207 mg, 0.5 mmol), NEt_3 (71 µl, 0.5 mmol) and nonanoyl chloride (92 µl, 0.5 mmol) in 5 ml DCM/abs according to the general procedure (PE/EtOAc 80/20 v/v) yielding **3.21a** (240 mg, 87 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.45 (m, 2H, C***H***$_2$NH), 2.70 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.39 (t, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 2H, $COCH_2$C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.26 (m, 10H, (C***H***$_2$)$_5$), 0.93 (m, 3H, CH_2C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 554.2 (MH^+, 100); $C_{27}H_{47}N_5O_5S$ (553.76).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-pentanoylguanidino]propyl}thiazol-2-yl-carbamate (3.33a)**

The title compound was prepared from **3.18** (160 mg, 0.4 mmol), NEt_3 (55 µl, 0.4 mmol) and pentanoyl chloride (47 µl, 0.4 mmol) in 4 ml DCM/abs according to the general procedure (PE/EtOAc 70/30 v/v) yielding **3.33a** (95 mg, 50 %) as pale yellow oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 484.1 (MH^+, 100); $C_{22}H_{37}N_5O_5S$ (483.62).

***tert*-Butyl 5-{3-[3-(*tert*-butoxycarbonyl)-2-nonanoylguanidino]propyl}thiazol-2-yl-carbamate (3.34a)**

The title compound was prepared from **3.18** (200 mg, 0.5 mmol), NEt_3 (71 µl, 0.5 mmol) and nonanoyl chloride (92 µl, 0.5 mmol) in 5 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-8 min: 90/10, 9-20 min: 80/20) yielded **3.34a** (130 mg, 60 %) as colorless oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 540.3 (MH^+, 100); $C_{26}H_{45}N_5O_5S$ (539.73).

3.5.1.5 Preparation of the deprotected N^G-acylated aminothiazolyl-propylguanidines 3.19-3.65

General procedure for the synthesis of deprotected acylguanidines 3.19-3.55, 3.64 and 3.65

TFA (20 %) was added to a solution of the protected acylguanidines **3.19-3.55**, **3.64** and **3.65** in DCM/abs, and the mixture was stirred at ambient temperature until the protecting groups were removed (3-5 h) (TLC control). Subsequently, the solvent was evaporated *in vacuo* and the residue was purified by preparative RP-HPLC. All compounds were obtained as trifluoroacetic acid salts.

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-propanoylguanidine (3.19)

Prepared from **3.19a** (170 mg, 0.36 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.19** (110 mg, 61 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49 (q, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.14 (t, ^3J = 7.41 Hz, 3H, COCH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 178.01 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 146.13 (quat. Thiaz-4-***C***), 118.44 (quat. Thiaz-5-***C***), 41.60 (-, ***C***H$_2$NH), 31.07 (-, CO***C***H$_2$), 29.71 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.63 (-, Thiaz-5-***C***H$_2$), 11.44 (+, Thiaz-4-***C***H$_3$), 8.57 (+, COCH$_2$***C***H$_3$); HREIMS: *m/z* for ([$C_{11}H_{19}N_5OS$]$^{+\bullet}$) calcd. 269.1310, found 269.1303; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 0.73 (t_R = 4.62 min, column B), purity = 99 %; $C_{11}H_{19}N_5OS \cdot 2TFA$ (497.41).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-pentanoylguanidine (3.20)

Prepared from **3.20a** (180 mg, 0.36 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.20** (100 mg, 53 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.41 Hz, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 2H, COCH$_2$C***H***$_2$), 1.38 (m, 2H, C***H***$_2$CH$_3$), 0.94 (t, ^3J = 7.41 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.41 (quat. ***C***=O), 170.97 (quat. Thiaz-2-***C***), 118.44 (quat. Thiaz-5-***C***), 41.61 (-, ***C***H$_2$NH), 37.52 (-, CO***C***H$_2$), 29.73 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.57 (-, COCH$_2$***C***H$_2$), 23.63 (-, Thiaz-5-***C***H$_2$), 23.12 (-, ***C***H$_2$CH$_3$), 14.05 (+, COCH$_2$***C***H$_3$), 11.44 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{13}H_{23}N_5OS$]$^{+\bullet}$) calcd.

297.1623, found 297.1623; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 2.96 (t_R = 7.91 min, column B), purity = 96 %; $C_{13}H_{23}N_5OS \cdot 2TFA$ (525.26).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-nonanoylguanidine (3.21)

Prepared from **3.21a** (230 mg, 0.42 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.21** (60 mg, 25 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 2H, COCH$_2$C***H***$_2$), 1.31 (m, 10H, (C***H***$_2$)$_5$), 0.90 (t, ^3J = 6.9 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.42 (quat. ***C***=O), 162.80 (quat. Thiaz-2-***C***), 155.31 (quat. ***C***=NH), 132.61 (quat. Thiaz-4-***C***), 118.44 (quat. Thiaz-5-***C***), 41.60 (-, ***C***H$_2$NH), 37.79 (-, CO***C***H$_2$), 33.00 (-, ***C***H$_2$), 30.37 (-, ***C***H$_2$), 30.29 (-, ***C***H$_2$), 30.04 (-, ***C***H$_2$), 29.74 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.49 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 23.62 (-, ***C***H$_2$CH$_3$), 14.45 (+, CH$_2$***C***H$_3$), 11.45 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ($[C_{17}H_{31}N_5OS]^{+\bullet}$) calcd. 353.2249, found 353.2247; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 4.06 (t_R = 13.54 min, column B), purity = 96 %; $C_{17}H_{31}N_5OS \cdot 2TFA$ (581.57).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-benzoylguanidine (3.22)

Prepared from **3.22a** (180 mg, 0.35 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.22** (130 mg, 68 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.00 (m, 2H, Ar-***H***), 7.75 (m, 1H, Ar-***H***), 7.70-7.55 (m, 2H, Ar-***H***), 3.50 (m, 2H, C***H***$_2$NH), 2.76 (m, 2H, Thiaz-5-C***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 171.27 (quat. ***C***=O), 135.27 (quat. Ar-***C***), 132.37 (quat. Thiaz-4-***C***), 130.19 (+, Ar-***C***), 129.32 (+, Ar-***C***), 118.40 (quat. Thiaz-5-***C***), 41.90 (-, ***C***H$_2$NH), 29.81 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.65 (-, Thiaz-5-***C***H$_2$), 11.48 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ($[C_{15}H_{19}N_5OS]^{+\bullet}$) calcd. 317.1310, found 317.1307; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.0 (t_R = 9.95 min, column A), purity = 98 %; $C_{15}H_{19}N_5OS \cdot 2TFA$ (545.17).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(2-phenylacetyl)guanidine (3.23)

Prepared from **3.23a** (200 mg, 0.38 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.23** (190 mg, 90 %) as yellow-brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.40-7.20 (m, 5H, Ar-***H***), 3.79 (s, 2H, COC***H***$_2$), 3.35 (m, 2H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-

$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.25 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 143.31 (quat. Ar-***C***), 132.58 (quat. Thiaz-4-***C***), 130.60 (+, Ar-***C***), 129.80 (+, Ar-***C***), 128.62 (+, Ar-***C***), 118.41 (quat. Thiaz-5-***C***), 44.42 (-, CO***C***H_2), 41.64 (-, ***C***H_2NH), 29.66 (-, Thiaz-5-CH_2***C***H_2), 23.59 (-, Thiaz-5-***C***H_2), 11.43 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{16}H_{21}N_5OS]^{+\bullet}$) calcd. 331.1467, found 331.1464; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.25 (t_R = 10.79 min, column A), purity = 100 %; $C_{16}H_{21}N_5OS \cdot 2TFA$ (559.48).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(3-phenylpropanoyl)guanidine (3.24)

Prepared from **3.24a** (180 mg, 0.33 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.24** (170 mg, 90 %) as yellow-brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.30-7.10 (m, 5H, Ar-***H***), 3.35 (m, 2H, C***H***$_2$NH), 2.96 (t, ^3J = 7.7 Hz, 2H, COC***H***$_2$), 2.79 (t, ^3J = 7.6 Hz, 2H, C***H***$_2$-Ar), 2.70 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.52 (quat. ***C***=O), 141.36 (quat. Ar-***C***), 132.60 (quat. Thiaz-4-***C***), 129.62 (+, Ar-***C***), 129.48 (+, Ar-***C***), 127.51 (+, Ar-***C***), 118.42 (quat. Thiaz-5-***C***), 41.59 (-, ***C***H_2NH), 39.52 (-, CO***C***H_2), 31.29 (-, ***C***H_2-Ar), 29.70 (-, Thiaz-5-CH_2***C***H_2), 23.60 (-, Thiaz-5-***C***H_2), 11.45 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{17}H_{23}N_5OS]^{+\bullet}$) calcd. 345.1623, found 345.1624; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.39 (t_R = 11.23 min, column A), purity = 99 %; $C_{17}H_{23}N_5OS \cdot 2TFA$ (573.5).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(4-phenylbutanoyl)guanidine (3.25)

Prepared from **3.25a** (200 mg, 0.36 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.25** (121 mg, 57 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.30-7.10 (m, 5H, Ar-***H***), 3.34 (m, 2H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.68 (m, 2H, C***H***$_2$-Ar), 2.48 (t, ^3J = 7.1 Hz, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.97 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.89 (m, 2H, C***H***$_2$$CH_2$-Ar); ^{13}C-NMR ($CD_3OD$) δ (ppm): 176.37 (quat. ***C***=O), 142.04 (quat. Ar-***C***), 132.63 (quat. Thiaz-4-***C***), 129.60 (+, Ar-***C***), 129.51 (+, Ar-***C***), 127.17 (+, Ar-***C***), 118.44 (quat. Thiaz-5-***C***), 41.62 (-, ***C***H_2NH), 37.11 (-, CO***C***H_2), 35.90 (-, ***C***H_2-Ar), 29.78 (-, Thiaz-5-CH_2***C***H_2), 27.14 (-, $COCH_2$***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 11.46 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{18}H_{25}N_5OS]^{+\bullet}$) calcd. 359.1780, found 359.1785; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.87 (t_R = 12.83 min, column A), purity = 100 %; $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.53).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(5-phenylpentanoyl)guanidine (3.26)

Prepared from **3.26a** (200 mg, 0.35 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.26** (189 mg, 90 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.29-7.10 (m, 5H, Ar-***H***), 3.34 (m, 2H, C***H***$_2$NH), 2.93 (m, 2H, Thiaz-5-C***H***$_2$), 2.63 (m, 2H, C***H***$_2$-Ar), 2.50 (m, 2H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$-Ar); ^{13}C-NMR (CD_3OD) δ (ppm): 177.26 (quat. ***C***=O), 163.79 (quat. Thiaz-2-***C***), 142.31 (quat. Ar-***C***), 132.63 (quat. Thiaz-4-***C***), 129.45 (+, Ar-***C***), 129.39 (+, Ar-***C***), 126.88 (+, Ar-***C***), 118.44 (quat. Thiaz-5-***C***), 41.60 (-, ***C***H$_2$NH), 37.59 (-, CO***C***H$_2$), 36.47 (-, ***C***H$_2$-Ar), 31.86 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.02 (-, COCH$_2$***C***H$_2$, ***C***H$_2$CH$_2$-Ar), 23.61 (-, Thiaz-5-***C***H$_2$), 11.45 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ($[C_{19}H_{27}N_5OS]^{+\bullet}$) calcd. 373.1936, found 373.1938; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.24 (t_R = 14.06 min, column A), purity = 99 %; $C_{19}H_{27}N_5OS \cdot 2TFA$ (601.56).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(6-phenylhexanoyl)guanidine (3.27)

Prepared from **3.27a** (200 mg, 0.34 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.27** (167 mg, 80 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.35-7.09 (m, 5H, Ar-***H***), 3.34 (m, 2H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.60 (m, 2H, C***H***$_2$-Ar), 2.45 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.62 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$-Ar), 1.37 (m, 2H, COCH$_2$CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.37 (quat. ***C***=O), 170.36 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 143.65 (quat. Ar-***C***), 132.59 (quat. Thiaz-4-***C***), 129.40 (+, Ar-***C***), 129.28 (+, Ar-***C***), 126.70 (+, Ar-***C***), 118.39 (quat. Thiaz-5-***C***), 41.55 (-, ***C***H$_2$NH), 37.66 (-, CO***C***H$_2$), 36.62 (-, ***C***H$_2$-Ar), 32.23 (-, ***C***H$_2$CH$_2$-Ar), 29.45 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.28 (-, ***C***H$_2$CH$_2$CH$_2$-Ar), 25.26 (-, COCH$_2$***C***H$_2$), 23.58 (-, Thiaz-5-***C***H$_2$), 11.41 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ($[C_{20}H_{29}N_5OS]^{+\bullet}$) calcd. 387.2093, found 387.2088; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.58 (t_R = 15.21 min, column A), purity = 95 %; $C_{20}H_{29}N_5OS \cdot 2TFA$ (615.58).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(3,3-diphenylpropanoyl)guanidine (3.28)

Prepared from **3.28a** (200 mg, 0.32 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.28** (62 mg, 30 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.29 (m, 8H, Ar-***H***), 7.17 (m, 2H, Ar-***H***), 4.59 (t, ^{3}J = 8.2 Hz, 1H, C***H***(Ar)$_2$), 3.27 (m, 2H, C***H***$_2$NH), 3.25 (m, 2H, COC***H***$_2$), 2.65 (t, ^{3}J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.13 (s,

3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.55 (quat. ***C***=O), 170.33 (quat. Thiaz-2-***C***), 144.49 (quat. Ar-***C***), 132.83 (quat. Thiaz-4-***C***), 129.86 (+, Ar-***C***), 129.70 (+, Ar-***C***), 129.57 (+, Ar-***C***), 129.16 (+, Ar-***C***), 128.81 (+, Ar-***C***), 127.80 (+, Ar-***C***), 118.36 (quat. Thiaz-5-***C***), 43.80 (-, CO***C***H$_2$), 41.49 (-, ***C***H$_2$NH), 29.61 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.51 (-, Thiaz-5-***C***H$_2$), 11.52 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{23}H_{27}N_5OS$]$^{+\bullet}$) calcd. 421.1936, found 421.1935; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.46 (t_R = 14.81 min, column A), purity = 100 %; $C_{23}H_{27}N_5OS \cdot 2TFA$ (649.6).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(cyclohexanecarbonyl)guanidine (3.29)

Prepared from **3.29a** (190 mg, 0.36 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.29** (188 mg, 95 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.42 (m, 1H, COC***H***), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.80 (m, 2H, cHex-***H***), 1.69 (m, 2H, cHex-***H***), 1.45 (m, 2H, cHex-***H***), 1.36 (m, 2H, cHex-***H***); ^{13}C-NMR (CD_3OD) δ (ppm): 180.18 (quat. ***C***=O), 170.35 (quat. Thiaz-2-***C***), 155.58 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.39 (quat. Thiaz-5-***C***), 43.78 (+, CO***C***H), 41.63 (-, ***C***H$_2$NH), 29.89 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.64 (-, cHex-***C***), 26.62 (-, cHex-***C***), 26.25 (-, cHex-***C***), 23.64 (-, Thiaz-5-***C***H$_2$), 11.41 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{15}H_{25}N_5OS$]$^{+\bullet}$) calcd. 323.1780, found 323.1778; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.42 (t_R = 11.34 min, column A), purity = 98 %; $C_{15}H_{25}N_5OS \cdot 2TFA$ (551.22).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(2-cyclohexylacetyl)guanidine (3.30)

Prepared from **3.30a** (150 mg, 0.28 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.30** (150 mg, 95 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 7.4 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.1 Hz, 2H, Thiaz-5-C***H***$_2$), 2.34 (d, ^3J = 6.9 Hz, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.93 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.80-1.60 (m, 5H, cHex-***H***), 1.40-1.17 (m, 6H, cHex-***H***); ^{13}C-NMR (CD_3OD) δ (ppm): 176.69 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 155.28 (quat. ***C***=NH), 132.61 (quat. Thiaz-4-***C***), 118.43 (quat. Thiaz-5-***C***), 48.75 (-, CO***C***H$_2$), 41.61 (-, ***C***H$_2$NH), 32.92 (+, cHex-***C***), 29.71 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.18 (-, cHex-***C***), 27.14 (-, cHex-***C***), 23.62 (-, Thiaz-5-***C***H$_2$), 11.45 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{16}H_{27}N_5OS$]$^{+\bullet}$) calcd. 337.1936, found

337.1930; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.85 (t_R = 12.77 min, column A), purity = 98 %; $C_{16}H_{27}N_5OS \cdot 2TFA$ (565.52).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-(11-aminoundecanoyl)guanidine (3.31)

Prepared from **3.31a** (50 mg, 0.07 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.31** (30 mg, 58 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.90 (t, ^3J = 7.41 Hz, 2H, C***H***$_2$NH$_2$), 2.71 (t, ^3J = 7.68 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.41 Hz, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$NH$_2$), 1.33 (m, 12H, (C***H***$_2$)$_6$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.49 (quat. ***C***=O), 170.39 (quat. Thiaz-2-***C***), 155.39 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.41 (quat. Thiaz-5-***C***), 41.57 (-, ***C***H$_2$NH), 40.77 (-, ***C***H$_2$NH$_2$), 37.77 (-, CO***C***H$_2$), 30.47 (-, ***C***H$_2$CH$_2$NH$_2$), 30.35 (-, ***C***H$_2$), 30.22 (-, ***C***H$_2$), 30.02 (-, ***C***H$_2$), 29.69 (-, Thiaz-5-CH$_2$***C***H$_2$), 28.62 (-, ***C***H$_2$), 27.47 (-, ***C***H$_2$CH$_2$CH$_2$NH$_2$), 25.51 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 11.44 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{19}H_{36}N_5OS$]$^{+\bullet}$) calcd. 396.2671, found 396.2683; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.94 (t_R = 7.89 min, column B), purity = 96 %; $C_{19}H_{36}N_6OS \cdot 3TFA$ (738.65).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-propanoylguanidine (3.32)

Prepared from **3.32a** (65 mg, 0.14 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.32** (25 mg, 37 %) as white amorphous solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.02 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49 (q, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.15 (t, ^3J = 7.4 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.97 (quat. ***C***=O), (quat. Thiaz-2-***C***), 155.34 (quat. ***C***=NH), (quat. Thiaz-5-***C***), 120,01 (+, Thiaz-4-***C***H), 41.50 (-, ***C***H$_2$NH), 31.08 (-, CO***C***H$_2$), 29.55 (-,Thiaz-5-CH$_2$***C***H$_2$), 24.96 (-, Thiaz-5-***C***H$_2$), 8.57 (+, CH$_2$***C***H$_3$); HREIMS: *m/z* for ([$C_{10}H_{17}N_5OS$]$^{+\bullet}$) calcd. 255.1154, found 255.1154; prep HPLC: MeCN/0.1 % TFA/aq (10/90-35/65); anal. HPLC: k`= 0.61 (t_R = 4.31 min, column B), purity = 100 %; $C_{10}H_{17}N_5OS \cdot 2TFA$ (483.38).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-pentanoylguanidine (3.33)

Prepared from **3.33a** (55 mg, 0.11 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.33** (10 mg, 18 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.78 (t, ^3J = 7.5 Hz,

2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 2H, C***H***$_2$CH$_3$), 1.39 (m, 2H, COCH$_2$C***H***$_2$), 0.94 (t, ^3J = 7.3 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.35 (quat. ***C***=O), (quat. Thiaz-2-***C***), (quat. ***C***=NH), 127.53 (quat. Thiaz-5-***C***), 122.57 (+, Thiaz-4-***C***H), 41.52 (-, ***C***H$_2$NH), 37.53 (-, CO***C***H$_2$), 29.52 (-,Thiaz-5-CH$_2$***C***H$_2$), 27.56 (-, COCH$_2$***C***H$_2$), 24.91 (-, Thiaz-5-***C***H$_2$), 23.13 (-, ***C***H$_2$CH$_3$), 14.06 (+, CH$_2$***C***H$_3$); HREIMS: *m/z* for ([$C_{12}H_{21}N_5OS$]$^{+\bullet}$) calcd. 283.1467, found 283.1469; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 1.74 (t_R = 7.34 min, column B), purity = 100 %; $C_{12}H_{21}N_5OS \cdot 2TFA$ (511.43).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-nonanoylguanidine (3.34)

Prepared from **3.34a** (60 mg, 0.11 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.34** (40 mg, 64 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.12 (s, 1H, Thiaz-4-***H***), 3.51 (m, 2H, C***H***$_2$NH), 2.88 (t, ^3J = 7.3 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.01 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.63 (m, 2H, COCH$_2$C***H***$_2$), 1.30 (m, 10H, (C***H***$_2$)$_5$), 0.87 (t, ^3J = 6.0 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.61 (quat. ***C***=O), 155.45 (quat. ***C***=NH), 125.42 (quat. Thiaz-5-***C***), 124.46 (+, Thiaz-4-***C***H), 40.85 (-, ***C***H$_2$NH), 37.10 (-, CO***C***H$_2$), 32.54 (-, ***C***H$_2$CH$_2$CH$_3$), 29.95 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.35 (-, ***C***H$_2$), 29.26 (-, ***C***H$_2$), 29.09 (-, ***C***H$_2$), 25.19 (-, COCH$_2$***C***H$_2$), 24.56 (-, Thiaz-5-**C**H$_2$), 23.29 (-, **C**H$_2$CH$_3$), 14.35 (+, CH$_2$**C**H$_3$); HREIMS: *m/z* for ([$C_{16}H_{29}N_5OS$]$^{+\bullet}$) calcd. 339.2093, found 339.2095; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.97 (t_R = 13.30 min, column B), purity = 100 %; $C_{16}H_{29}N_5OS \cdot 2TFA$ (567.54).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-nonadecanoylguanidine (3.35)

Prepared from **3.35a** (50 mg, 0.07 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.35** (15 mg, 30 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.36 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.30 (m, 30H, (C***H***$_2$)$_{15}$), 0.89 (t, ^3J = 6.9 Hz, 3H, CH$_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.40 (quat. ***C***=O), 154.34 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.71 (+, Thiaz-4-***C***H), 41.50 (-, ***C***H$_2$NH), 37.80 (-, CO***C***H$_2$), 33.12 (-, ***C***H$_2$CH$_2$CH$_3$), 30.81 (-, ***C***H$_2$), 30.74 (-, ***C***H$_2$), 30.60 (-, ***C***H$_2$), 30.52 (-, ***C***H$_2$), 30.40 (-, ***C***H$_2$), 30.04 (-, ***C***H$_2$), 29.54 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.48 (-, COCH$_2$***C***H$_2$), 24.90 (-, Thiaz-5-***C***H$_2$), 23.78 (-, ***C***H$_2$CH$_3$), 14.48 (+, CH$_2$***C***H$_3$); HREIMS: *m/z* for ([$C_{26}H_{49}N_5OS$]$^{+\bullet}$) calcd. 479.3658,

found 479.3655; prep HPLC: MeCN/0.1 % TFA/aq (40/60-70/30); anal. HPLC: k`= 7.80 (t_R = 23.53 min, column B), purity = 93 %; $C_{26}H_{49}N_5OS \cdot 2TFA$ (707.81).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(4-phenylbutanoyl)guanidine (3.36)

Prepared from **3.36a** (180 mg, 0.33 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.36** (160 mg, 85 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.22 (m, 5H, Ar-***H***), 7.01 (s, 1H, Thiaz-4-***H***), 3.36 (t, ^3J = 7.35 Hz, 2H, C***H***$_2$NH), 2.76 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.67 (t, ^3J = 7.7 Hz, 2H, COC***H***$_2$), 2.48 (t, ^3J = 7.4 Hz, 2H, C***H***$_2$-Ar), 1.96 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$, COCH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.07 (quat. ***C***=O), 166.27 (quat. Thiaz-2-***C***), 142.57 (quat. Ar-***C***), 129.59 (+, 2 Ar-***C***H), 129.50 (+, 2 Ar-***C***H), 127.16 (+, Ar-***C***H), 126.23 (quat. Thiaz-5-***C***), 123.36 (+, Thiaz-4-***C***H), 41.49 (-, ***C***H$_2$NH), 37.12 (-, CO***C***H$_2$), 35.90 (-, Ar-***C***H$_2$), 29.49 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.13 (-, COCH$_2$***C***H$_2$), 24.89 (-, Thiaz-5-***C***H$_2$); HREIMS: *m/z* for ([$C_{17}H_{23}N_5OS$]$^{+\bullet}$) calcd. 345.1623, found 345.1624; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.95 (t_R = 9.60 min, column B), purity = 99 %; $C_{17}H_{23}N_5OS \cdot 2TFA$ (573.5).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(5-phenylpentanoyl)guanidine (3.37)

Prepared from **3.37a** (100 mg, 0.18 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.37** (40 mg, 38 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.22 (m, 2H, Ar-***H***), 7.14 (m, 2H, Ar-***H***), 7.01 (s, 1H, Thiaz-4-***H***), 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.76 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.63 (m, 2H, C***H***$_2$Ar), 2.48 (m, 2H, COC***H***$_2$) 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.68 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$Ar); ^{13}C-NMR (CD_3OD) δ (ppm): 177.26 (quat. ***C***=O), 143.31 (quat. Ar-***C***), 129.45 (+, Ar-***C***), 129.38 (+, Ar-***C***), 126.87 (+, Ar-***C***), 41.48 (-, ***C***H$_2$NH), 37.58 (-, Ar-***C***H$_2$), 36.47 (-, CO***C***H$_2$), 31.84 (-, Ar-CH$_2$***C***H$_2$), 29.50 (-,Thiaz-5-CH$_2$***C***H$_2$), 25.00 (-, COCH$_2$***C***H$_2$), 24.92 (-,Thiaz-5-***C***H$_2$); HREIMS: *m/z* for ([$C_{18}H_{25}N_5OS$]$^{+\bullet}$) calcd. 359.1780, found 359.1781; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.28 (t_R = 11.44 min, column B), purity = 100 %; $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.53).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(2-cyclohexyl-2-phenylacetyl)guanidine (3.38)

Prepared from **3.38a** (210 mg, 0.35 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.38** (150 mg, 68 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.37-7.26 (m, 5H, Ar-***H***), 6.99 (s, 1H, Thiaz-4-C***H***), 3.38 (m, 1H, C***H***(Ar)cHex), 3.31 (m, 2H, C***H***$_2$NH), 2.73 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.13 (m, 1H, cHex-***H***),

1.92 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.85-1.60 (m, 4H, cHex-C***H***), 1.41-1.10 (m, 6H, cHex-***H***); ^{13}C-NMR (CD_3OD) δ (ppm): 177.74 (quat. ***C***=O), 171.80 (quat. Thiaz-2-***C***), 155.24 (quat. ***C***=NH), 138.14 (quat. Ar-***C***), 129.84 (+, Ar-***C***), 129.74 (+, Ar-***C***), 128.92 (+, Ar-***C***), 126.33 (quat. Thiaz-5-***C***), 123.27 (+, Thiaz-4-***C***H), 61.35 (+, ***C***H(Ar)cHex), 41.82 (+, cHex-***C***), 41.55 (-, ***C***H_2NH), 32.97 (-, cHex-***C***), 31.20 (-, cHex-***C***), 29.39 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 27.35 (-, cHex-***C***), 27.07 (-, cHex-***C***), 27.00 (-, cHex-***C***), 24.88 (-, Thiaz-5-***C***H_2); HREIMS: *m/z* for ($[C_{21}H_{29}N_5OS]^{+\bullet}$) calcd. 399.2093, found 399.2096; prep HPLC: MeCN/0.1 % TFA/aq (30/70-60/40); anal. HPLC: k`= 3.97 (t_R = 13.31 min, column B), purity = 100 %; $C_{21}H_{29}N_5OS \cdot 2TFA$ (627.59).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(2-methyl-3-phenylpropanoyl)guanidine (3.39)

Prepared from **3.39a** (90 mg, 0.16 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.39** (20 mg, 22 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.30-7.14 (m, 5H, Ar-***H***), 6.99 (s, 1H, Thiaz-4-***H***), 3.33 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.99 (dd, ^2J = 12.7 Hz, ^3J = 7.6 Hz, 1H, Ar-C***H***HCH), 2.89 (m, 1H, COC***H***(CH_3)CH_2), 2.72 (m, 3H, Thiaz-5-C***H***$_2$, Ar-CH***H***CH), 1.92 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.18 (d, ^3J = 6.6 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 180.27 (quat. ***C***=O), 155.13 (quat. ***C***=NH), 140.02 (quat. Ar-***C***), 130.14 (+, Ar-***C***), 129.54 (+, Ar-***C***), 127.68 (+, Ar-***C***), 126.33 (quat. Thiaz-5-***C***), 123.38 (+, Thiaz-4-***C***H), 45.02 (+, CO***C***HCH_3), 41.46 (-, ***C***H_2NH), 40.63 (-, Ar-***C***H_2), 29.44 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 24.88 (-, Thiaz-5-***C***H_2), 17.04 (+, CH***C***H_3); HREIMS: *m/z* for ($[C_{17}H_{23}N_5OS]^{+\bullet}$) calcd. 345.1623, found 345.1625; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.73 (t_R = 9.98 min, column B), purity = 99 %; $C_{17}H_{23}N_5OS \cdot 2TFA$ (573.5).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(2-benzylbutanoyl)guanidine (3.40)

Prepared from **3.40a** (35 mg, 0.06 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.40** (27 mg, 77 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.22 (m, 5H, Ar-***H***), 7.12 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.96 (m, 2H, Ar-C***H***$_2$), 2.85 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.75 (m, 1H, COC***H***), 2.00 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.77-1.45 (m, 2H, C***H***$_2CH_3$), 0.90 (t, ^3J = 7.4 Hz, 3H, $CH_2C\boldsymbol{H}_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 180.02 (quat. ***C***=O), 155.53 (quat. ***C***=NH), 139.93 (quat. Ar-***C***), 129.93 (+, Ar-***C***), 129.15 (+, Ar-***C***), 127.21 (+, Ar-***C***), 125.29 (quat. Thiaz-5-***C***), 123.97 (+, Thiaz-4-***C***H), 51.03 (+, CO***C***H), 40.89 (-, ***C***H_2NH), 38.66 (-, Ar-***C***H_2), 29.14 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 25.30 (-, ***C***H_2CH_3), 24.56 (-, Thiaz-5-***C***H_2), 11.83 (+,

CH***C***H_3); HREIMS: *m/z* for ([$C_{18}H_{25}N_5OS$]$^{+\bullet}$) calcd. 359.1780, found 359.1787; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.07 (t_R = 10.89 min, column B), purity = 100 %; $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.53).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(3-*p*-tolylbutanoyl)guanidine (3.41)

Prepared from **3.41a** (70 mg, 0.13 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.41** (31 mg, 41 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.10 (m, 4H, Ar-***H***), 6.98 (s, 1H, Thiaz-4-***H***), 3.33 (m, 2H, C***H***$_2$NH), 3.23 (m, 1H, C***H***CH$_3$), 2.73 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 2.27 (s, 3H, Ar-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.28 (d, ^3J = 7.0 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.12 (quat. ***C***=O), 171.83 (quat. Thiaz-2-***C***), 155.18 (quat. ***C***=NH), 143.31 (quat. Ar-***C***), 137.27 (quat. Ar-***C***-CH$_3$), 130.22 (+, Ar-***C***H), 127.29 (+, Ar-***C***H), 126.31 (quat, Thiaz-5-***C***), 123.33 (+, Thiaz-4-***C***H), 46.35 (-, CO***C***H$_2$), 41.41 (-, ***C***H$_2$NH), 37.37 (+, ***C***HCH$_3$), 29.43 (-, Thiaz-5-CH$_2$***C***H$_2$), 24.83 (-, Thiaz-5-***C***H$_2$), 22.33 (+, Ar-C-***C***H$_3$), 21.07 (+, CH***C***H$_3$); HREIMS: *m/z* for ([$C_{18}H_{25}N_5OS$]$^{+\bullet}$) calcd. 359.1780, found 359.1788; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.14 (t_R = 11.09 min, column B), purity = 99 %; $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.53).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(4-hydroxyphenylpropanoyl)guanidine (3.42)

Prepared from **3.42a** (100 mg, 0.18 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.42** (25 mg, 24 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.04 (d, ^3J = 8.5 Hz, 2H, Ar-***H***), 6.99 (s, 1H, Thiaz-4-***H***), 6.69 (d, ^3J = 8.5 Hz, 2H, Ar-***H***), 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.87 (t, ^3J = 7.1 Hz, C***H***$_2$-Ar), 2.73 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.94 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.65 (quat. ***C***=O), 157.05 (quat. Ar-***C***-OH), 132.01 (quat. Ar-***C***), 130.44 (+, 2 Ar-***C***), 123.46 (+, Thiaz-4-***C***), 116.32 (+, 2 Ar-***C***), 41.47 (-, ***C***H$_2$NH), 39.99 (-, CO***C***H$_2$), 30.62 (-, Ar-***C***H$_2$), 29.49 (-, Thiaz-5-CH$_2$***C***H$_2$), 24.87 (-, Thiaz-5-***C***H$_2$); HREIMS: *m/z* for ([$C_{16}H_{21}N_5O_2S$]$^{+\bullet}$) calcd. 347.1416, found 347.1416; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.54 (t_R = 6.80 min, column B), purity = 100 %; $C_{16}H_{21}N_5O_2S \cdot 2TFA$ (575.48).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(6-amino-3-phenylhexanoyl)guanidine (3.43)

Prepared from **3.43a** (40 mg, 0.06 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.40** (20 mg, 46 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.33-7.18 (m, 5H, Ar-***H***), 6.98 (s, 1H, Thiaz-4-***H***), 3.28 (m, 2H, C***H***$_2$NH), 3.18

(m, 1H, CH_2CH), 2.85 (m, 4H, $COCH_2$, CH_2NH_2), 2.71 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-CH_2), 1.90 (m, 2H, Thiaz-5-CH_2CH_2), 1.76 (m, 2H, $CH_2CH_2CH_2NH_2$), 1.50 (m, 2H, $CH_2CH_2NH_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.77 (quat. ***C***O), 169.64 (quat. Thiaz-2-***C***), 155.15 (quat. ***C***=NH), 143.69 (quat. Ar-***C***), 129.85 (+, 2 Ar-***C***), 128.82 (+, 2 Ar-***C***), 128.17 (+, Ar-***C***), 126.29 (quat. Thiaz-5-***C***), 123.40 (+, Thiaz-4-***C***), 44.82 (-, CH_2NH_2), 42.88 (+, CH_2CH), 41.32 (-, CH_2NH), 40.56 (-, $COCH_2$), 33.82 (-, $CH_2CH_2CH_2NH_2$), 29.41 (-, Thiaz-5-CH_2CH_2), 26.64 (-, $CH_2CH_2NH_2$), 24.80 (-, Thiaz-5-CH_2); HREIMS: *m/z* for ($[C_{19}H_{28}N_6OS]^{+\bullet}$) calcd. 389.2118, found 389.2121; prep HPLC: MeCN/0.1 % TFA/aq (10/90-35/65); anal. HPLC: k`= 1.04 (t_R = 5.46 min, column B), purity = 97 %; $C_{19}H_{28}N_6OS \cdot 2TFA$ (730.59).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(3-methyl-4-phenylbutanoyl)guanidine (3.44)

Prepared from **3.44a** (60 mg, 0.10 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.44** (50 mg, 85 %) as white amorphous solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.25 (m, 5H, Ar-***H***), 7.14 (s, 1H, Thiaz-4-***H***), 3.51 (m, 2H, CH_2NH), 2.88 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-CH_2), 2.71 (dd, ^2J = 13.2 Hz, ^3J = 5.8 Hz, 1H, Ar-CHHCH), 2.51 (m, 2H, COC***H***HCH, CH_3), 2.35 (m, 2H, Ar-CH***H***CH, COCH***H***CH), 2.01 (m, 2H, Thiaz-5-CH_2CH_2), 0.92 (d, ^3J = 6.2 Hz, 3H, $CHCH_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.89 (quat. ***C***=O), 155.38 (quat. ***C***=NH), 141.23 (quat. Ar-***C***), 130.08 (+, Ar-***C***), 129.07 (+, Ar-***C***), 126.88 (+, Ar-***C***), 125.32 (quat. Thiaz-5-***C***), 123.75 (+, Thiaz-4-***C***H), 44.10 (-, Ar-CH_2), 43.46 (-, $COCH_2$), 40.84 (-, CH_2NH), 33.02 (+, $CHCH_3$), 29.09 (-, Thiaz-5-CH_2CH_2), 24.56 (-, Thiaz-5-CH_2), 19.51 (+, $CHCH_3$); HREIMS: *m/z* for ($[C_{18}H_{25}N_5OS]^{+\bullet}$) calcd. 359.1780, found 359.1786; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.10 (t_R = 10.96 min, column B), purity = 99 %; $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.53).

1[3-(2-Aminothiazol-5-yl)propyl]-2-[4-(3-methoxyphenyl)-3-methylbutanoyl]-guanidine (3.45)

Prepared from **3.45a** (50 mg, 0.08 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.45** (27 mg, 55 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.16 (m, 1H, Ar-***H***), 7.00 (s, 1H, Thiaz-4-***H***), 6.74 (m, 3H, Ar-***H***), 3.75 (s, 3H, OCH_3), 3.34 (t, ^3J = 7.0 Hz, 2H, CH_2NH), 2.76 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-CH_2), 2.65-2.25 (m, 5H, $COCH_2$, Ar-CH_2, $CHCH_3$), 1.94 (m, 2H, Thiaz-5-CH_2CH_2), 0.97 (d, ^3J = 6.2 Hz, 3H, $CHCH_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.78 (quat. ***C***=O), 171.83 (quat.

Thiaz-2-***C***), 161.20 (quat. Ar-***C***(3)), 155.18 (quat. ***C***=NH), 142.95 (quat. Ar-1-***C***)), 130.33 (+, Ar-5-***C***), 126.34 (quat, Thiaz-5-***C***), 123.33 (+, Thiaz-4-***C***H), 122.74 (+, Ar-6-***C***), 115.95 (+, Ar-2-***C***), 112.68 (+, Ar-4-***C***), 55.59 (+, O***C***H_3), 44.55 (-, Ar-***C***H_2), 43.99 (-, CO***C***H_2), 41.45 (-, ***C***H_2NH), 33.32 (+, C***H***CH_3), 29.47 (-, Thiaz-5-CH_2***C***H_2), 24.89 (-, Thiaz-5-***C***H_2), 20.10 (+, CH***C***H_3); HREIMS: *m/z* for ($[C_{19}H_{27}N_5O_2S]^{+\bullet}$) calcd. 389.1885, found 389.1886; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.15 (t_R = 11.09 min, column B), purity = 99 %; $C_{19}H_{27}N_5O_2S$ · 2TFA (617.55).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-[4-(4-methoxyphenyl)-3-methylbutanoyl]-guanidine (3.46)

Prepared from **3.46a** (60 mg, 0.10 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.46** (49 mg, 79 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.07 (d, ^3J = 8.6 Hz, 2H, Ar-***H***), 7.00 (s, 1H, Thiaz-4-***H***), 6.80 (d, ^3J = 8.6 Hz, 2H, Ar-***H***), 3.74 (s, 3H, OC***H***$_3$), 3.33 (m, 2H, C***H***$_2$NH), 2.75 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.57-2.21 (m, 5H, COC***H***$_2$, Ar-C***H***$_2$, C***H***CH_3), 1.93 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.96 (d, ^3J = 6.2 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.90 (quat. ***C***=O), 171.85 (quat. Thiaz-2-***C***), 159.65 (quat. Ar-4-***C***), 155.21 (quat. ***C***=NH), 133.29 (quat. Ar-1-***C***), 131.36 (+, Ar-***C***), 126.32 (quat. Thiaz-5-***C***), 123.31 (+, Thiaz-4-***C***H), 114.74 (+, Ar-***C***), 55.69 (+, O***C***H_3), 44.57 (-, Ar-***C***H_2), 43.16 (-, CO***C***H_2), 41.43 (-, ***C***H_2NH), 33.64 (+, C***H***CH_3), 29.46 (-, Thiaz-5-CH_2***C***H_2), 24.89 (-, Thiaz-5-***C***H_2), 20.14 (+, CH***C***H_3); HREIMS: *m/z* for ($[C_{19}H_{27}N_5O_2S]^{+\bullet}$) calcd. 389.1885, found 389.1887; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.11 (t_R = 10.99 min, column B), purity = 100 %; $C_{19}H_{27}N_5O_2S$ · 2TFA (617.55).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(2-cyclohexylacetyl)guanidine (3.47)

Prepared from **3.47a** (70 mg, 0.13 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.47** (43 mg, 60 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.33 (d, ^3J = 6.9 Hz, 2H, COC***H***$_2$), 1.96 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.82-1.63 (m, 6H, cHex-C***H***), 1.27 (m, 3H, cHex-C***H***), 1.04 (m, 2H, cHex-C***H***); ^{13}C-NMR (CD_3OD) δ (ppm): 176.66 (quat. ***C***=O), 155,63 (quat. ***C***=NH), 126.16 (quat. Thiaz-5-***C***), 123.43 (+, Thiaz-4-***C***H), 45.53 (-, CO***C***H_2), 41.52 (-, ***C***H_2NH), 36.02 (+, cHex-***C***), 33.92 (-, cHex-***C***), 29.50 (-, Thiaz-5-CH_2***C***H_2), 27.18 (-, cHex-***C***), 27.14 (-, cHex-***C***), 24.90 (-, Thiaz-5-***C***H_2); HREIMS: *m/z* for ($[C_{15}H_{25}N_5OS]^{+\bullet}$) calcd. 323.1780, found

323.1786; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.79 (t_R = 10.13 min, column B), purity = 100 %; $C_{15}H_{25}N_5OS \cdot 2TFA$ (551.5).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(3-cyclohexylpropanoyl)guanidine (3.48)

Prepared from **3.48a** (90 mg, 0.17 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.48** (38 mg, 40 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.48 (t, ^3J = 7.7 Hz, 2H, COC***H***$_2$) 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.78-1.63 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.55 (m, 2H, C***H***$_2$-cHex), 1.25 (m, 4H, cHex-C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.57 (quat. ***C***=O), 158,35 (quat. Thiaz-2-***C***), 126.40 (quat. Thiaz-5-***C***), 123.82 (+, Thiaz-4-***C***H), 41.55 (-, ***C***H$_2$NH), 38.42 (-, CO***C***H$_2$), 35.49 (-, COCH$_2$***C***H$_2$), 34.14 (-, cHex-***C***), 32.87 (+, cHex-***C***), 29.56 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.62 (-, cHex-***C***), 27.35 (-, cHex-***C***), 24.90 (-, Thiaz-5-***C***H$_2$); HREIMS: *m/z* for ([$C_{16}H_{27}N_5OS$]$^{+\bullet}$) calcd. 337.1935, found 337.1936; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.34 (t_R = 11.60 min, column B), purity = 99 %; $C_{16}H_{27}N_5OS \cdot 2TFA$ (565.52).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(4-cyclohexylbutanoyl)guanidine (3.49)

Prepared from **3.49a** (170 mg, 0.31 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.49** (150 mg, 83 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.77 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.44 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$) 1.96 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.78-1.60 (m, 7H, cHex-***H***), 1.40 (m, 4H, C***H***$_2$CH$_2$-cHex, cHex-***H***), 1.23 (m, 4H, C***H***$_2$-cHex, cHex-***H***); ^{13}C-NMR (CD_3OD) δ (ppm): 177.36 (quat. ***C***=O), 155,15 (quat. ***C***=NH), 126.37 (quat. Thiaz-5-***C***), 123.29 (+, Thiaz-4-***C***H), 41.51 (-, ***C***H$_2$NH), 38.73 (-, CO***C***H$_2$), 38.06 (-, ***C***H$_2$-cHex), 37.83 (+, cHex-***C***), 34.41 (-, cHex-***C***), 29.48 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.77 (-, cHex-***C***), 27.46 (-, cHex-***C***), 24.89 (-, Thiaz-5-***C***H$_2$), 22.88 (-, COCH$_2$***C***H$_2$); HREIMS: *m/z* for ([$C_{17}H_{29}N_5OS$]$^{+\bullet}$) calcd. 351.2093, found 351.2088; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.66 (t_R = 12.46 min, column B), purity = 100 %; $C_{17}H_{29}N_5OS \cdot 2TFA$ (579.55).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(3-cyclohexyl-5-methylhexanoyl)guanidine (3.50)

Prepared from **3.50a** (60 mg, 0.10 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.50** (30 mg, 48 %) as white amorphous solid. ^{1}H-NMR

(CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.0 Hz, 2H, C***H***$_2$NH), 2.76 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49 (dd, ^2J = 15.6 Hz, ^3J = 7.0 Hz, 1H, COC***H***HCH), 2.29 (dd, ^2J = 15.6 Hz, ^3J = 7.0 Hz, 1H, COCH***H***CH), 1.96 (m, 3H, Thiaz-5-CH$_2$C***H***$_2$, C***H***$(CH_3)_2$), 1.81-1.50 (m, 6H, cHex-***H***, C***H***-cHex), 1.43-1.14 (m, 6H, cHex-***H***), 1.06 (m, 2H, C***H***$_2$CH$(CH_3)_2$), 0.89 (d, ^3J = 3.1 Hz, 3H, CHC***H***$_3$), 0.87 (d, ^3J = 3.1 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.68 (quat. ***C***=O), 171.79 (quat. Thiaz-2-***C***), (quat. ***C***=NH), 126.34 (quat. Thiaz-5-***C***), 123.66 (+, Thiaz-4-***C***H), 42.02 (+,***C***H-cHex), 41.81 (-, ***C***H$_2$CH$(CH_3)_2$), 41.50 (-, ***C***H$_2$NH), 40.26 (-, CO***C***H$_2$), 38.84 (+, cHex-***C***), 30.98 (-, cHex-***C***), 30.11 (-, cHex-***C***), 29.54 (-,Thiaz-5-CH$_2$***C***H$_2$), 27.97 (-, cHex-***C***), 27.91 (-, cHex-***C***), 27.83 (-, cHex-***C***), 26.69 (+, ***C***H$(CH_3)_2$), 24.87 (-, Thiaz-5-***C***H$_2$), 23.35 (+, CH***C***H$_3$), 22.98 (+, CH***C***H$_3$); HREIMS: *m/z* for ($[C_{20}H_{35}N_5OS]^{+\bullet}$) calcd. 393.2562, found 393.2563; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 4.77 (t_R = 15.44 min, column B), purity = 99 %; $C_{20}H_{35}N_5OS \cdot 2TFA$ (621.63).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(4-cyclohexyl-3-methylbutanoyl)guanidine (3.51)

Prepared from **3.51a** (40 mg, 0.07 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.51** (15 mg, 25 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.36 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.76 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.44 (dd, ^2J = 14.7 Hz, ^3J = 8.1 Hz, 1H, COC***H***HCH), 2.22 (dd, ^2J = 14.7 Hz, ^3J = 8.1 Hz, 1H, COCH***H***CH), 2.11 (m, 1H, C***H***CH$_3$), 1.95 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.78-1.61 (m, 6H, cHex-***H***), 1.38-1.04 (m, 7H, cHex-***H***, cHexC***H***$_2$), 0.93 (d, ^3J = 6.4 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.95 (quat. ***C***=O), 171.61 (quat. Thiaz-2-***C***), 155.28 (quat. ***C***=NH), 126.35 (quat. Thiaz-5-***C***), 123.65 (+, Thiaz-4-***C***H), 45.80 (-, ***C***H$_2$-cHex), 45.58 (-, CO***C***H$_2$), 41.51 (-, ***C***H$_2$NH), 36.10 (+, cHex-***C***H), 35.12 (-, cHex-***C***H$_2$), 34.14 (-, cHex-***C***H$_2$), 29.52 (-, Thiaz-5-CH$_2$***C***H$_2$), 28.30 (+, ***C***HCH$_3$), 27.78 (-, cHex-***C***H$_2$), 27.48 (-, cHex-***C***H$_2$), 27.40 (-, cHex-***C***H$_2$), 24.89 (-, Thiaz-5-***C***H$_2$), 20.07 (+, CH***C***H$_3$); HREIMS: *m/z* for ($[C_{18}H_{31}N_5OS]^{+\bullet}$) calcd. 365.2249, found 365.2247; prep HPLC: MeCN/0.1 % TFA/aq (25/75-50/50); anal. HPLC: k`= 4.15 (t_R = 13.78 min, column B), purity = 99 %; $C_{18}H_{31}N_5OS \cdot 2TFA$ (593.58).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-[3-(cyclohexylmethyl)pentanoyl]guanidine (3.52)

Prepared from **3.52a** (50 mg, 0.09 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.52** (25 mg, 46 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 6.98 (s, 1H, Thiaz-4-***H***), 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.74 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.35 m, 2H, COC***H***$_2$), 1.95 (m, 3H, Thiaz-5-CH_2C***H***$_2$, C***H***CH_2CH_3), 1.76-1.57 (m, 5H, cHex-***H***), 1.43-1.03 (m, 10H, cHex-***H***, C***H***$_2$-cHex, C***H***$_2CH_3$), 0.89 (d, ^3J = 7.4 Hz, 3H, CHC***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.31 (quat. ***C***=O), 171.79 (quat. Thiaz-2-***C***), 155.29 (quat. ***C***=NH), 126.32 (quat. Thiaz-5-***C***), 123.40 (+, Thiaz-4-***C***H), 42.64 (-, ***C***H_2-cHex, CO***C***H_2), 41.45 (-, ***C***H_2NH), 36.13(+, ***C***HCH_2CH_2), 34.78 (-, cHex-***C***), 34.68 (-, cHex-***C***), 34.25 (+, cHex-***C***), 29.49 (-, Thiaz-5-CH_2***C***H_2), 27.75 (-, ***C***H_2CH_3), 27.53 (-, cHex-***C***), 27.44 (-, cHex-***C***), 24.86 (-, Thiaz-5-***C***H_2), 10.90 (+, CH_2***C***H_3); HREIMS: *m/z* for ($[C_{19}H_{33}N_5OS]^{+\bullet}$) calcd. 379.2406, found 379.2407; prep HPLC: MeCN/0.1 % TFA/aq (25/75-50/50); anal. HPLC: k`= 4.47 (t_R = 14.63 min, column B), purity = 100 %; $C_{19}H_{33}N_5OS \cdot 2TFA$ (607.6).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(6-aminohexanoyl)guanidine (3.53)

Prepared from **3.53a** (400 mg, 0.65 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.53** (350 mg, 82 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.36 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.92 (t, ^3J = 7.6 Hz, 2H, C***H***$_2NH_2$), 2.77 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.52 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.95 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.64 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2CH_2NH_2$), 1.46 (m, 2H, $COCH_2CH_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.28 (quat. ***C***=O), 126.34 (quat. Thiaz-5-***C***), 123.31 (+, Thiaz-4-***C***H), 41.43 (-, ***C***H_2NH), 40.51 (-, ***C***H_2NH_2), 37.33 (-, CO***C***H_2), 29.49 (-, Thiaz-5-CH_2***C***H_2), 28.25 (-, ***C***$H_2CH_2NH_2$), 26.73 (-, $COCH_2CH_2$***C***H_2), 24.88 (-, Thiaz-5-***C***H_2), 24.75 (-, $COCH_2$***C***H_2); HREIMS: *m/z* for ($[C_{13}H_{24}N_6OS]^{+\bullet}$) calcd. 312.1732, found 312.1726; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 0.28 (t_R = 3.42 min, column B), purity = 98 %; $C_{13}H_{24}N_6OS \cdot 3TFA$ (654.49).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(11-aminoundecanoyl)guanidine (3.54)

Prepared from **3.54a** (80 mg, 0.12 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.54** (40 mg, 46 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.36 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.90 (t, ^3J = 7.9 Hz, 2H, C***H***$_2NH_2$), 2.76 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.41 Hz, 2H, COC***H***$_2$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.64 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2CH_2NH_2$), 1.32 (m, 12H, (C***H***$_2)_6$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.47 (quat. ***C***=O), 171,85 (quat. Thiaz-2-***C***),

155.38 (quat. ***C***=NH), 126.33 (quat. Thiaz-5-***C***), 123.32 (+, Thiaz-4-***C***H), 41.44 (-, ***C***H_2NH), 40.77 (-, ***C***H_2NH_2), 37.77 (-, CO***C***H_2), 30.44 (-, ***C***$H_2CH_2NH_2$), 30.33 (-, ***C***H_2), 30.21 (-, ***C***H_2), 30.01 (-, ***C***H_2), 29.47 (-, Thiaz-5-CH_2***C***H_2), 28.60 (-, $COCH_2CH_2$***C***H_2), 27.46 (-, ***C***$H_2CH_2CH_2NH_2$), 25.49 (-, $COCH_2$***C***H_2), 24.98 (-, Thiaz-5-***C***H_2); HREIMS: *m/z* for ($[C_{18}H_{34}N_6OS]^{+\bullet}$) calcd. 382.2515, found 382.2514; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 1.83 (t_R = 7.56 min, column B), purity = 100 %; $C_{18}H_{34}N_6OS \cdot 3TFA$ (724.63).

S-6-{3-[3-(2-aminothiazol-5-yl)propyl]guanidin-2-yl}-6-oxohexyl ethanethioate (3.55)
Prepared from **3.55a** (250 mg, 0.44 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.55** (220 mg, 83 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.87 (t, ^3J = 7.2 Hz, 2H, SC***H***$_2$), 2.77 (t, ^3J = 7.2 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.3 Hz, 2H, COC***H***$_2$), 2.30 (s, 3H, COC***H***$_3$), 1.95 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.69 (m, 2H, SCH_2C***H***$_2$), 1.58 (m, 2H, $COCH_2$C***H***$_2$), 1.46 (m, 2H, $COCH_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.15 (quat. ***C***=O), 126.38 (quat. Thiaz-5-***C***), 123.53 (+, Thiaz-4-***C***), 41.51 (-, ***C***H_2NH), 37.53 (-, CO***C***H_2), 30.52 (+, CO***C***H_3), 30.47 (-, S***C***H_2), 29.57 (-, Thiaz-5-CH_2-***C***H_2), 28.98 (-, SCH_2***C***H_2), 24.90 (-, $COCH_2$***C***H_2, SCH_2CH_2***C***H_2), 24.84 (-, Thiaz-5-***C***H_2); ES-MS ($DCM/MeOH + NH_4OAc$) *m/z* (%): 206.9 ($(M+2H)^{2+}$+MeCN, 100), 372 (MH^+, 50); HRLSIMS: *m/z* for ($[C_{15}H_{25}N_5O_2S_2 + H]^+$) calcd. 372.1522, found 372.1523; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.48 (t_R = 9.30 min, column B), purity = 98 %; $C_{15}H_{25}N_5O_2S_2 \cdot 2TFA$ (599.56).

3-(2-Amino-4-methylthiazol-5-yl)propylguanidine (3.64)
Prepared from **3.17** (80 mg, 0.19 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.65** (56 mg, 67 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.21 (t, ^3J = 6.8 Hz, 2H, C***H***$_2$NH), 2.68 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.83 (m, 2H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.47 (quat. ***C***=O), 170.39 (quat. Thiaz-2-***C***), 155.39 (quat. ***C***=NH), 132.61 (quat. Thiaz-4-***C***), 118.43 (quat. Thiaz-5-***C***), 41.61 (-, ***C***H_2NH), 29.74 (-, Thiaz-5-CH_2***C***H_2), 23.84 (-, Thiaz-5-***C***H_2), 11.45 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_8H_{15}N_5S]^{+\bullet}$) calcd. 213.1048, found 213.1048; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 0.27 (t_R = 3.41 min, column B), purity = 95 %; $C_8H_{15}N_5S \cdot 2TFA$ (441.34).

3-(2-Aminothiazol-5-yl)propylguanidine (3.65)

Prepared from **3.18** (90 mg, 0.22 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **3.65** (70 mg, 75 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 6.98 (s, 1H, Thiaz-4-***H***), 3.24 (t, ^3J = 7.5 Hz, 2H, C***H***$_2$NH), 2.73 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 1.88 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.47 (quat. ***C***=O), 170.72 (quat. Thiaz-2-***C***), 155.38 (quat. ***C***=NH), 126.49 (quat. Thiaz-5-***C***), 123.14 (+, Thiaz-4-***C***H), 41.36 (-, ***C***H$_2$NH), 30.30 (-, Thiaz-5-CH$_2$***C***H$_2$), 24.79 (-, Thiaz-5-***C***H$_2$); HREIMS: *m/z* for ([$C_7H_{13}N_5S$]$^{+\bullet}$) calcd. 199.0892, found 199.0892; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 0.27 (t_R = 3.40 min, column B), purity = 100 %; $C_7H_{13}N_5S$ · 2TFA (427.32).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-(6-sulfanylhexanoyl)guanidine (3.56)

1 N NaOH (1 ml) was added to a solution of **3.55** (200 mg, 0.33 mmol) in MeCN (2 ml) and the mixture was stirred for 30 min. After neutralization with 1 N HCl the solvent was removed under reduced pressure and the product purified with preparative RP-HPLC to give **3.56** (49 mg, 27 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.0 Hz, 2H, C***H***$_2$NH), 2.76 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.69 (t, ^3J = 7.1 Hz, 2H, SC***H***$_2$), 2.49 (t, ^3J = 7.3 Hz, 2H, COC***H***$_2$), 1.96 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.79-1.60 (m, 4H, SCH$_2$C***H***$_2$, COCH$_2$C***H***$_2$), 1.46 (m, 4H, C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.23 (quat. ***C***=O), 171.77 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.51 (+, Thiaz-4-***C***), 41.49 (-, ***C***H$_2$NH), 39.25 (-, S***C***H$_2$), 37.98 (-, CO***C***H$_2$), 29.77 (-, SCH$_2$***C***H$_2$), 29.48 (-, Thiaz-5-CH$_2$-***C***H$_2$), 28.67 (-, SCH$_2$CH$_2$***C***H$_2$), 24.99 (-, COCH$_2$***C***H$_2$), 24.89 (-, Thiaz-5-***C***H$_2$); HRLSIMS: *m/z* for ([$C_{13}H_{23}N_5OS_2$ + H]$^+$) calcd. 330.1422, found 330.1422; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.17 (t_R = 8.49 min, column B), purity = 97 %; $C_{13}H_{23}N_5OS_2$ · 2TFA (557.52).

General procedure for the preparation of compounds 3.57, 3.59, 3.62 and 3.63

NEt_3 (3 eq) was added to a solution of **3.31**, **3.53** or **3.71**[10] (1 eq) in MeCN. Subsequently, a solution of succinimidyl 4-F-benzoate or propionate (0.8 eq), respectively, in MeCN was added and stirred for 4-5 h at room temperature. The solvent was removed under reduced pressure and the product purified by preparative RP-HPLC.

1-[3-(2-Aminothiazol-5-yl)propyl]-2-[6-(4-fluorobenzoylamino)-3-phenyl hexanoyl]-guanidine (3.57)

The title compound was prepared from **3.71**[10] (23 mg, 31 µmol) in 1.5 ml MeCN, succinimidyl 4-F-benzoate (6 mg, 25 µmol) in 0.5 ml MeCN and NEt_3 (13 µl, 93 µmol) according to the general procedure yielding **3.57** (25 mg, 75 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.81 (m, 2H, Ar-***H***), 7.22 (m, 7H, Ar-***H***), 3.33 (m, 2H, C***H***$_2$NH), 3.26 (m, 2H, C***H***$_2$NHCO), 3.19 (m, 1H, CH_2C***H***), 2.82 (m, 2H, COC***H***$_2$), 2.65 (m, 2H, Thiaz-5-C***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.89-1.70 (m, 4H, Thiaz-5-CH_2C***H***$_2$, C***H***$_2$$CH_2$CHNH), 1.48 (m, 2H, C***H***$_2$$CH_2$$CH_2$NH); ^{13}C-NMR ($CD_3OD$) δ (ppm): 175.96 (quat. ***C***=O), 170.48 (quat. Thiaz-2-***C***), 169.06 (quat. ***C***=O), 155.09 (quat. ***C***=NH), 144.37 (quat. Ar-***C***), 132.58 (quat. Ar-***C***), 130.89 (+,Ar-***C***), 130.77 (+, Ar-***C***), 129.72 (+, Ar-***C***), 128.76 (+, Ar-***C***), 127.93 (+, Ar-***C***), 118.38 (quat. Thiaz-5-***C***), 116.51 (+, Ar-***C***), 116.22 (+, Ar-***C***), 45.06 (+, CH_2***C***H), 42.93 (-, ***C***H_2NHCO), 41.52 (-, CO***C***H_2), 40.56 (-, ***C***H_2NH), 34.40 (-, ***C***$H_2$$CH_2$$CH_2$$NH_2$), 29.63 (-, Thiaz-5-$CH_2$***C***$H_2$), 28.38 (-, ***C***$H_2$$CH_2$$NH_2$), 23.53 (-, Thiaz-5-***C***H_2), 11.45 (+,Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{27}H_{33}FN_6O_2S]^{+\bullet}$) calcd. 524.2370, found 524.2376; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.49 (t_R = 12.02 min, column B), purity = 99 %; $C_{27}H_{33}FN_6O_2S$ · 2TFA (752.68).

1-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-2-[11-(propionylamino)undecanoyl]-guanidine (3.59)

The title compound was prepared from **3.31** (23 mg, 31 µmol) in 1.5 ml MeCN, NHS-propionate (4.3 mg, 25 µmol) in 0.5 ml MeCN and NEt_3 (13 µl, 93 µmol) according to the general procedure yielding **3.59** (10 mg, 75 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.36 (m, 2H, C***H***$_2$NH), 3.14 (t, ^3J = 7. 1 Hz, 2H, C***H***$_2$NHCO), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.18 (m, 5H, COC***H***$_2$$CH_3$, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-$CH_2$C***H***$_2$), 1.65 (m, 2H, $COCH_2$C***H***$_2$), 1.48 (m, 2H, C***H***$_2$$CH_2$NH), 1.31 (m, 12H, (C***H***$_2$)$_6$), 1.11 (t, ^3J = 7.6 Hz, 2H, CH_2C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.38 (quat. ***C***=O), 170.40 (quat. Thiaz-2-***C***), 155.47 (quat. ***C***=NH), 132.71 (quat. Thiaz-4-***C***), 118.46 (quat. Thiaz-5-***C***), 41.62 (-, ***C***H_2NH), 40.39 (-, ***C***H_2NHCO), 37.79 (-, CO***C***H_2), 30.60 (-, ***C***$H_2$$CH_2$NHCO), 30.45 (-, ***C***$H_2$$CH_3$), 30.37 (-, ***C***$H_2$), 30.34 (-, ***C***$H_2$), 30.27 (-, ***C***$H_2$), 30.00 (-, Thiaz-5-$CH_2$***C***$H_2$), 27.98 (-, ***C***$H_2$$CH_2$$CH_2$NH), 25.47 (-, $COCH_2$***C***H_2), 23.61 (-, Thiaz-5-***C***H_2), 11.49 (+, Thiaz-4-***C***H_3), 10.67 (+, CH_2***C***H_3); HREIMS: *m/z* for ($[C_{22}H_{40}N_6O_2S]^{+\bullet}$) calcd. 492.2933, found 492.2943; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.19 (t_R = 11.21 min, column B), purity = 98 %; $C_{22}H_{40}N_6O_2S$ · 2TFA (680.7).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-[6-(propionylamino)hexanoyl]guanidine (3.62)

The title compound was prepared from **3.53** (34 mg, 52 µmol) in 1.5 ml MeCN, NHS-propionate (7 mg, 42 µmol) in 0.5 ml MeCN and NEt_3 (22 µl, 160 µmol) according to the general procedure yielding **3.62** (23 mg, 74 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.3 Hz, 2H, C***H***$_2$NH), 3.16 (m, 2H, C***H***$_2$NHCO), 2.79 (m, 2H, Thiaz-5-C***H***$_2$), 2.48 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.18 (q, ^3J = 7.6 Hz, 2H, COC***H***$_2$$CH_3$), 1.96 (m, 2H, Thiaz-5-$CH_2$C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2$$CH_2$NHCO), 1.35 (m, 2H, $COCH_2CH_2$C***H***$_2$), 1.11 (t, ^3J = 7.6 Hz, 3H, $COCH_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.15 (quat. ***C***=O), 126.38 (quat. Thiaz-5-***C***), 123.47 (+, Thiaz-4-***C***H), 41.55 (-, ***C***H_2NH), 40.06 (-, ***C***H_2NHCO), 37.65 (-, CO***C***H_2), 30.27 (-, ***C***H_2CH_2NHCO), 30.12 (-, ***C***H_2CH_3), 29.53 (-,Thiaz-5-CH_2***C***H_2), 27.27 (-, $COCH_2CH_2$***C***H_2), 25.02 (-, $COCH_2$***C***H_2), 24.92 (-, Thiaz-5-***C***H_2), 10.63 (+, CH_2***C***H_3); HREIMS: *m/z* for ($[C_{16}H_{28}N_6O_2S]^{+\bullet}$) calcd. 368.1994, found 368.1993; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.18 (t_R = 5.84 min, column B), purity = 85 %; $C_{16}H_{28}N_6O_2S$ · 2TFA (596.54).

1-[3-(2-Aminothiazol-5-yl)propyl]-2-[6-(4-fluorobenzoylamino)hexanoyl]guanidine (3.63)

The title compound was prepared from **3.53** (44 mg, 67 µmol) in 1.5 ml MeCN, NHS-4-F-benzoate (13 mg, 54 µmol) in 0.5 ml MeCN and NEt_3 (38 µl, 0.2 mmol) according to the general procedure yielding **3.63** (14 mg, 39 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.85 (m, 2H, Ar-***H***), 7.17 (t, ^3J = 8.7 Hz, 2H, Ar-***H***), 7.00 (s, 1H, Thiaz-4-***H***), 3.36 (m, 4H, C***H***$_2$NH, C***H***$_2$NHCO), 2.76 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 1.94 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.68 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2$$CH_2$NHCO), 1.43 (m, 2H, C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.22 (quat. ***C***=O), 164.51 (quat. Ar-***C***-F), 162.39 (quat. Thiaz-2-***C***), 155.29 (quat. ***C***=NH), 130.89 (+, Ar-***C***), 130.78 (+, Ar-***C***), 126.36 (quat. Thiaz-5-***C***), 123.36 (+, Thiaz-4-***C***H), 116.53 (+, Ar-***C***), 116.23 (+, Ar-***C***), 41.51 (-, ***C***H_2NH), 40.74 (-, ***C***H_2NHCO), 37.63 (-, CO***C***H_2), 30.16 (-, ***C***H_2CH_2NHCO), 29.49 (-, Thiaz-5-CH_2***C***H_2), 27.29 (-, $COCH_2CH_2$***C***H_2), 25.06 (-, $COCH_2$***C***H_2), 24.91 (-, Thiaz-5-***C***H_2); HREIMS: *m/z* for ($[C_{20}H_{27}FN_6O_2S]^{+\bullet}$) calcd. 434.1900, found 434.1900; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.33 (t_R = 8.90 min, column B), purity = 100 %; $C_{20}H_{27}FN_6O_2S$ · 2TFA (662.57).

General procedure for the preparation of the fluorescent compounds 3.58 and 3.60

To a solution of **3.31** or **3.71**[10] (2.5 eq) in MeCN was added NEt_3 (7.5 eq). Subsequently, a solution of py-1[12] ((*E*)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)-ethenyl]-2,6-dimethylpyrylium tetrafluoroborate, 1 eq) in DMF was added. After 1-2 min the color changed from blue to red. The reaction was stopped by addition of 10 % TFA/aq after an incubation period of 1 h at room temperature. The product was purified by preparative RP-HPLC.

1-(6-{Amino[3-(2-amino-4-methylthiazol-5-yl)propylamino]methyleneamino}-6-oxo-4-phenylhexyl)-4-[(*E*)-2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)ethenyl]-2,6-dimethylpyridinium trifluoroacetate (3.58)

The title compound was prepared from **3.71**[10] (4.59 mg, 6.2 µmol) in 0.8 ml MeCN, NEt_3 (2.6 µl, 18.5 µmol) and py-1 (0.97 mg. 2.5 µmol) in50 µl DMF according to the general procedure affording **3.58** (1.5 mg, 58 %) as red oil. ES-MS (MeOH + 0.1 % FAc) *m/z* (%): 345.6 ($(M+2H)^{2+}$, 100); prep. HPLC: MeCN/0.1 % TFA/aq (40/60-60/40); anal. HPLC: k`= 4.66 (t_R = 15.15 min, column B), purity = 97 %; $[C_{41}H_{52}N_7OS]^+ \cdot 3TFA$ (1033.02).

1-(11-{Amino[3-(2-amino-4-methylthiazol-5-yl)propylamino]methyleneamino}-11-oxoundecyl)-4-[(*E*)-2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)ethenyl]-2,6-dimethylpyridinium trifluoroacetate (3.60)

The title compound was prepared from **3.31** (4.7 mg, 6.4 µmol) in 0.8 ml MeCN, NEt_3 (2.7 µl, 19 µmol) and py-1 (1.0 mg. 2.5 µmol) in 50 µl DMF according to the general procedure affording **3.60** (1.6 mg, 62 %) as red oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 342.7 ($(M+2H)^{2+}$, 100); HPLC: MeCN/0.1 % TFA/aq (40/60-60/40); anal. HPLC: k`= 4.92 (t_R = 15.84 min, column B), purity = 96 %; $[C_{40}H_{58}N_7OS]^+ \cdot 3TFA$ (1027.06).

(*E*)-1-[6-(11-{Amino[3-(2-amino-4-methylthiazol-5-yl)propylamino]methylene-amino}-11-oxoundecylamino)-6-oxohexyl]-2-{(2*E*,4*E*)-5-[3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indolium-2-yl]penta-2,4-dienylidene}-3,3-dimethylindoline-5-sulfonate (3.61)

NEt_3 (4.1 µl, 15 µmol) was added to a solution of **3.31** (4.8 mg, 3.3 µmol) in 0.8 ml MeCN. Subsequently, a solution of NHS-S0586 (1.16 mg, 1.5 µmol) in 0.1 ml DMF was added and stirred overnight at room temperature. The reaction was stopped by addition of

10 % TFA/aq (15 µl). The product was purified by preparative RP-HPLC (MeCN/0.1 % TFA/aq (30/70-70/30)) yielding **3.61** (1.3 mg, 67 %) as blue oil. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 532.3 ($(M+2H)^{2+}$, 100), 1063.7 (MH^+, 10); prep. HPLC: MeCN/0.1 % TFA/aq (30/70-70/30); anal. HPLC: k`= 2.15 (t_R = 8.43 min, column B), purity = 97 %; $[C_{54}H_{77}N_8O_8S_3]^-$ · TFA (1176.45).

3.5.2 Pharmacological methods

3.5.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). [γ-^{32}P]GTP and [γ-^{33}P]GTP, respectively, were synthesized according to a previously described method.[25] [^{32}P]P_i (8,500 – 9,100 Ci/mmol orthophosphoric acid) and [^{33}P]P_i (3,000 Ci/mmol orthophosphoric acid) were purchased from Hartmann Analytics GmbH (Braunschweig, Germany). All unlabeled nucleotides, glycerol-3-phosphate dehydrogenase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche (Mannheim, Germany). 3-Phosphoglycerate kinase and L-α-glycerol phosphate were from Sigma-Aldrich Chemie GmbH (München, Germany). Unlabeled GTPγS was from Roche (Mannheim, Germany) and [^{35}S]GTPγS was from Hartmann Analytics GmbH (Braunschweig, Germany). GF/B filters were from Brandel (Gaithersburg, MD, USA).

3.5.2.2 Determination of histamine receptor agonism and antagonism in GTPase assays

Generation of recombinant baculoviruses, cell culture and membrane preparation

Recombinant baculoviruses encoding human H_1R, or a fusion protein of the human H_2R with $Gs\alpha_S$, or a fusion protein of the guinea pig H_2R with $Gs\alpha_S$, or the human H_3R or a fusion protein of the human H_4R with RGS19 were prepared as described,[13,26-28] using the BaculoGOLD transfection kit (BDPharmingen, San Diego, CA) according to the manufacturer's instructions.

Sf9 cells were cultured in 250 or 500 ml disposable Erlenmeyer flasks at 28 °C under rotation at 150 rpm in Insect-Xpress medium (Lonza, Velviers, Belgium) supplemented with 5 % (v/v) fetal calf serum (Biochrom, Berlin, Germany) and 0.1 mg/ml gentamicin

(Lonza, Walkersville, MD). Cells were maintained at a density of 0.5 – 6.0 x 10^6 cells/ml. After initial transfection, high-titer virus stocks were generated by two sequential virus amplifications. In the first amplification, cells were seeded at 2.0 x 10^6 cells/ml and infected with a 1:100 dilution of the supernatant from the initial transfection. Cells were cultured for 7 days, resulting in the lysis of the entire cell population. The supernatant was harvested and stored under light protection at 4 °C. In a second amplification, cells were seeded at 3.0 x 10^6 cells/ml and infected with a 1:20 dilution of the supernatant fluid from the first amplification. Cells were cultured for 48 h, and the supernatant was harvested. After a 48 h culture period, the majority of cells showed signs of infections (e.g. altered morphology, viral inclusion bodies), whereas most of the cells were still intact. The supernatant fluid from the second amplification was stored under light protection at 4 °C and used as routine virus stock for membrane preparations. For membrane preparation, cells were sedimented by centrifugation (1000 rpm, 5 min, rt) and suspended in fresh medium at 3.0 x 10^6 cells/ml. Cells were infected with 1:100 dilutions of high-titer baculovirus stocks encoding the various histamine receptors, histamine receptor fusion proteins, G-protein subunits and RGS proteins. Cells were cultured for 48 h before membrane preparation. Sf9 membranes were prepared as described,[29] using 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 10 µg/ml benzamidine and 10 µg/ml leupeptin as protease inhibitors. Membranes were suspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4) and stored at -80 °C until use. Protein concentrations were determined using the DC protein assay kit (Bio-Rad, München, Germany).

Steady-state GTPase activity assay with Sf9 insect cell membranes expressing histamine H_1-H_4 receptors

Membranes were thawed, sedimented and resuspended in 10 mM Tris/HCl, pH 7.4. In the case of the H_1R and H_2R, Sf9 membranes expressing either H_1R isoforms plus RGS4 or H_2R-G$s\alpha_S$ fusion proteins, respectively, were used.[13,21] H_3R-regulated GTP hydrolysis was determined with membranes co-expressing human H_3R, mammalian Gα_{i2}, G$\beta_{1\gamma2}$ and RGS4. Human H_4R activity was measured with membranes co-expressing an H_4R-RGS19 fusion protein with Gα_{i2} and G$\beta_1\gamma_2$. Assay tubes contained Sf9 membranes (10-20 µg of protein/tube), $MgCl_2$ (H_1R, H_2R: 1.0 mM; H_3R, H_4R: 5.0 mM), 100 µM EDTA, 100 µM ATP, 100 nM GTP, 100 µM adenylyl imidodiphosphate, 5 mM creatine phosphate, 40 µg creatine kinase and 0.2 % (w/v) bovine serum albumin in 50 mM Tris/HCl, pH 7.4,

as well as ligands at various concentrations. In H_4R assays, NaCl (final concentration of 100 mM) was included. Reaction mixtures (80 µl) were incubated for 2 min at 25 °C before the addition of 20 µl [γ-^{32}P]GTP (0.1 µCi/tube) or [γ-^{33}P]GTP (0.05 µCi/tube). Reactions were run for 20 min at 25 °C and terminated by the addition of 900 µl of slurry consisting of 5% (w/v) activated charcoal suspended in 50 mM NaH_2PO_4, pH 2.0. Charcoal absorbs nucleotides but not P_i. Charcoal-quenched reaction mixtures were centrifuged for 7 min at room temperature at 13.000 *g*. 600 µl of the supernatant fluid were removed and $^{32}P_i$ or $^{33}P_i$ was determined by Cerenkov or liquid scintillation counting, respectively. Enzyme activities were corrected for spontaneous hydrolysis of [γ-^{32}P]GTP or [γ-^{33}P]GTP, respectively, determined in tubes containing all components described above, plus a high concentration of unlabeled GTP (1 mM) to prevent enzymatic hydrolysis of the labeled nucleotides in the presence of Sf9 membranes. Spontaneous [γ-^{32}P]GTP or [γ-^{33}P]GTP degradation was <1 % of the total amount of radioactivity added. The experimental conditions chosen ensured that not more than 10% of the total amount of added [γ-^{32}P]GTP and [γ-^{33}P]GTP was converted to $^{32}P_i$ and $^{33}P_i$, respectively. All experimental data were analyzed by non-linear regression with the Prism 5 program (GraphPad Software, San Diego, CA).

3.5.2.3 Histamine H_2 receptor assay on isolated guinea pig right atrium

Guinea pigs of either sex (250-500 g) were stunned by a blow on the neck and exsanguinated. The heart was rapidly removed, and the right atrium was quickly dissected and set up isometrically in Krebs-Henseleit`s solution under a diastolic resting force of approximately 5 mN in a jacketed 20 ml organ bath of 32.5 °C as previously described.[14] The bath fluid (composition [mM]: NaCl 118.1, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.64, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.0, sodium pyruvate 2.0) was equilibrated with 95% O_2 - 5% CO_2 and additionally contained (*RS*)-propanolol (0.3 µM) to block β-adrenergic receptors. Stock solutions (10 mM) and all dilutions of ligands (1, 0.1 and 0.01 mM) were made in freshly prepared bath fluid instead of distilled water in order to prevent absorption at glass surfaces. Experiments were started after 30 min of continuous washing and an additional equilibration period of 15 min. Two successive curves for histamine displayed a significant desensitization of 0.13 ± 0.02 (N = 16 control organs). This value was used to correct each individual experiment. *Agonists:* Two successive concentration-frequency curves were established: the first to histamine (0.1-30 µM) and the second for

the agonist under study in the absence or presence of cimetidine (10 μM, 30 min incubation time). Furthermore, the sensitivity to 30, 100 or 300 μM cimetidine was routinely checked at the end of each H_2R agonist concentration-effect curve and a significant reduction of frequency was observed. Relative potency of the agonist under study was calculated from the corrected pEC_{50} difference. pEC_{50} values are given relative to the long term mean value for histamine (pEC_{50} = 6.00) in our laboratory (pEC_{50} = 6.00 + ΔpEC_{50}).

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Chapter 4

Homobivalent acylguanidines: twin compounds as histamine H_2 receptor agonists

4.1 Introduction

As demonstrated in chapter 3, the structure-activity relationships of N^G-acylated 3-(2-aminothiazol-5-yl)propylguanidines (cf. Figure 4.1) revealed that even space-filling substituents at the guanidine group are well tolerated.[1] This prompted us to explore the applicability of the bivalent ligand approach, based on the working hypothesis that such compounds should possess increased H_2R agonistic potency and might be useful to study hypothetical H_2R dimers.

Over the last few decades the understanding of GPCR structure and function has been challenged by the discovery that GPCRs are able to form homo- and hetero-oligomeric complexes.[2-4] Meanwhile, the existence of homo- and hetero-dimers has been demonstrated for several class A GPCRs including opioid receptors,[5-7] adrenergic receptors,[8] somatostatin receptors,[9-10] dopaminergic receptors,[11-13] muscarinergic receptors[14-15] as well as the histamine receptor subtypes.[16-20] The term bivalent ligand is widely used and refers to molecules containing two sets of pharmacophoric entities linked through a spacer.[21] The design of bivalent ligands requires considerations of various aspects, including the choice of the initial monomeric lead compound, the choice of an appropriate attachment point for the spacer and the choice of length and chemical composition of the spacer.[22] Bivalent ligands are thought to exhibit a greater potency than that corresponding to double concentration of a monovalent ligand.[3,21] This concept has been studied for various GPCRs,[22] for instance, for opioid receptors,[23] in more detail. The

bivalent ligand approach has proven to be promising to improve potency and selectivity but also the pharmacokinetic profile of compounds.[24]

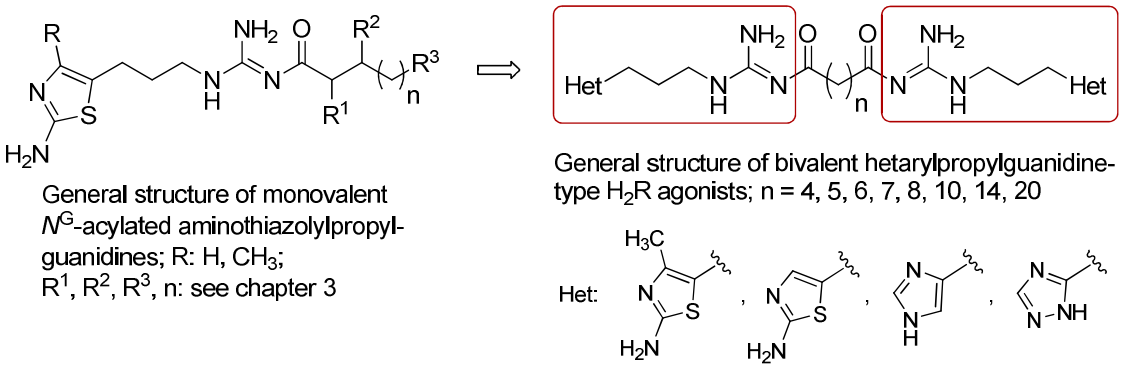


Figure 4.1. Overview of structural modifications resulting in the bivalent title compounds.

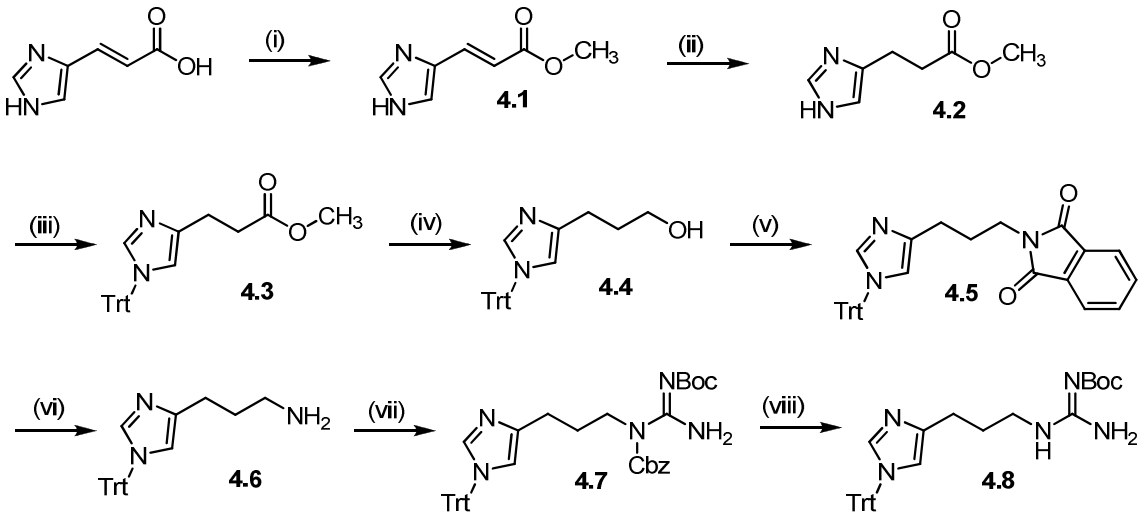
For opioid receptors, the distance between two recognition sites of a contact dimer with a TM5/TM6 interface is about 22 to 27 Å as suggested from molecular modeling.[3] In an approach to explore the structural requirements of putative bivalent H_2R agonists, we synthesized and pharmacologically investigated bivalent ligands ("twin compounds") with two hetarylpropylguanidine entities, linked at the N^G-nitrogen atoms with dicarboxylic acids as spacers with lengths between 6 and 27 Å.

4.2 Chemistry

The bivalent acylguanidine-type compounds were preferentially synthesized by analogy with the procedure developed for the N^G-acylation of monovalent hetarylpropyl-guanidines as described in chapter 3,[1,25] using two equivalents of mono Boc-protected hetarylpropylguanidines **3.17**, **3.18** and **4.8** and one equivalent of the pertinent dicarboxylic acids. The synthetic strategies aimed at compounds of maximal purity on the low mg scale rather than at the optimization of yields and synthetic routes.

The required Boc-protected *N*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine building block **4.8** was synthesized with minor modifications as previously described starting from urocanic acid (Scheme 4.1).[25] After esterification, hydrogenation of the double bond and trityl-protection of the imidazole-NH, the ester group was reduced with $LiAlH_4$ to obtain the alcohol **4.4**. Conversion of the alcohol functionality to the primary amine **4.6** was accomplished under *Mitsunobu* conditions[26] via the phthalimide **4.5** and subsequent

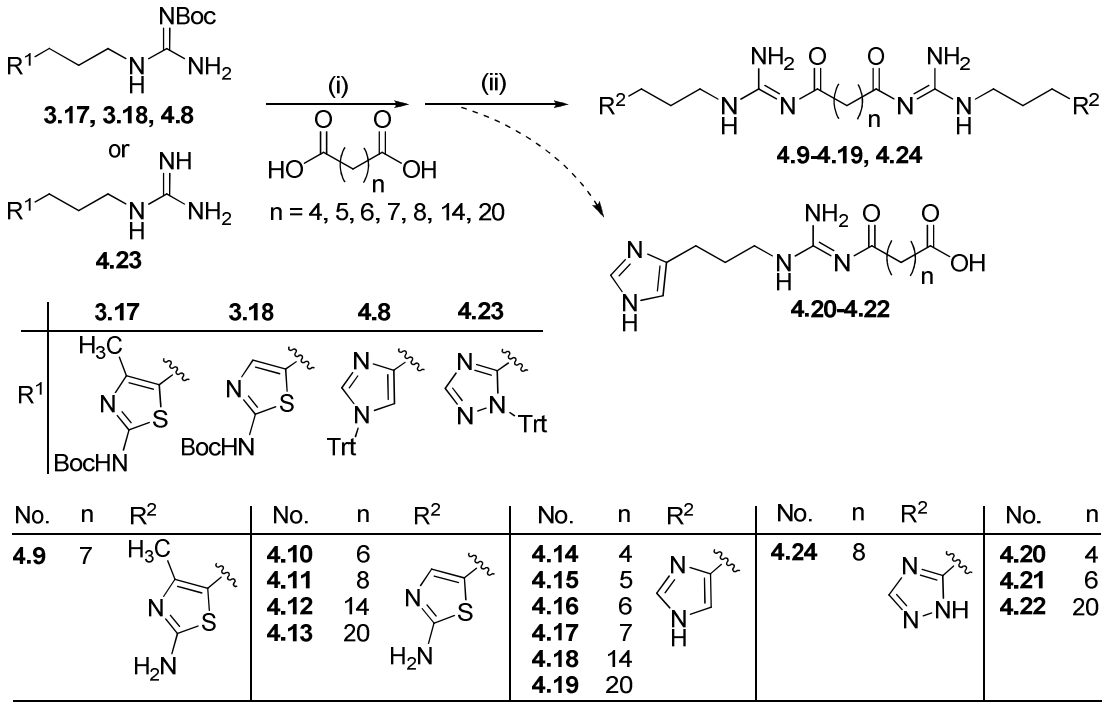
treatment with hydrazine monohydrate. The free amine **4.6** was then coupled to the guanidinylating reagent **3.3** by analogy with the procedure described for the aminothiazoles in chapter 3.[1] Finally, the Cbz-group was removed by hydrogenation to yield the N^{G}-Boc-,N^{Im}-Trt-protected imidazolylpropylguanidine **4.8**.



Scheme 4.1. Synthesis of the imidazolylpropylguanidine building block **4.8**. Reagents and conditions: (i) anhydrous Na_2SO_4, H_2SO_4/conc., MeOH/abs, 30 h, reflux;[27] (ii) H_2, Pd/C (10 %) cat., MeOH, 5 bar, 24 h, rt; (iii) CPh_3Cl (1.1 eq), NEt_3 (2.8 eq), MeCN, 12 h, rt; (iv) $LiAlH_4$ (2 eq), THF/abs, Et_2O/abs, 2 h, reflux; (v) phthalimide (1 eq), PPh_3 (1 eq), DIAD (1 eq), THF/abs, 24 h, rt; (vi) $N_2H_2{\cdot}H_2O$ (5 eq), EtOH, 1 h, reflux; (vii) **3.3** (1 eq), $HgCl_2$ (2 eq), NEt_3 (3 eq), DCM/abs, 48 h, rt; (viii) H_2, Pd/C (10 %), MeOH/THF (1:1), 8 bar, 6-7 d, rt.

To obtain the designated symmetrical bivalent ligands **4.9-4.19**, the mono Boc-protected hetarylpropylguanidines **3.17**, **3.18** and **4.8** were coupled to alkanedioic acids of various length using EDAC, HOBt and DIEA as standard coupling reagents to yield the protected acylguanidines **4.9a-4.19a**. Thereby, the Boc-protected guanidine (**3.17**, **3.18**, **4.8**), at its terminal position (N^{G}), reacts similarly to amines but at lower reaction rates. Finally, removal of the protecting groups under acidic conditions gave the symmetrical bivalent acylguanidines **4.9-4.19** (Scheme 4.2), which were purified by preparative RP-HPLC. In certain cases, mono-acylated side products were able to be separated during the purification step *via* preparative RP-HPLC. In this way, low amounts of the mono-acylated imidazolylpropylguanidines **4.20-4.22** with one free carboxylic function were obtained. In addition, the bivalent N^{G}-acylated 1,2,4-triazol-5-ylpropylguanidine **4.24** was synthesized starting from the Trt-protected triazolylpropylguanidine building block **4.23** (cf. Scheme 4.2), which was recently prepared in our workgroup.[28] In contrast to the aforementioned acylation steps, the guanidine building block **4.23** was deprotonated with

NaH and coupled to decanedioic acid, which was activated by CDI, to yield the Trt-protected precursor **4.24a**. Treatment with TFA/aq (20 %) and purification with preparative RP-HPLC gave N^1,N^{10}-Bis{[3-(1*H*-1,2,4-triazol-5-yl)propylamino](amino)-methylene}decanediamide (**4.24**) in high purity.



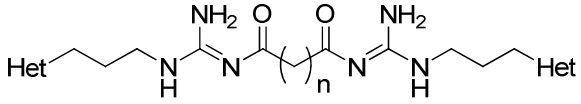
No.	n	R^2	No.	n	R^2	No.	n	R^2	No.	n	R^2	No.	n
4.9	7		**4.10** **4.11** **4.12** **4.13**	6 8 14 20		**4.14** **4.15** **4.16** **4.17** **4.18** **4.19**	4 5 6 7 14 20		**4.24**	8		**4.20** **4.21** **4.22**	4 6 20

Scheme 4.2. General procedure for the preparation of the symmetrical bivalent acylguanidines **4.9**-**4.19** and **4.24** and the mono-acylated imidazolylpropylguanidines **4.20**-**4.22**. Reagents and conditions: (i) for **4.9**-**4.19**: EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 16 h, rt; for **4.24**: CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF/abs, 3-4 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt. Compound **4.23** was provided by Dr. P. Igel. For experimental data see Ref.[28]

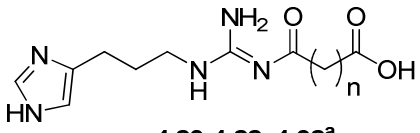
4.3 Pharmacological results and discussion

In addition to the newly synthesized acylguanidines **4.9**-**4.22** and **4.24**, previously prepared bivalent H_2R agonists[29] are included in this section to a more comprehensive overview of the structure-activity relationships of this class of compounds. Table 4.1 gives a structural overview of all investigated ligands.

Table 4.1. Structural overview of investigated twin compounds (**4.9**-**4.19**, **4.24** and **4.25**-**4.31**) and related N^G-acylated imidazolylpropylguanidines (**4.20**-**4.22** and **4.32**).



4.9-4.19, 4.24, 4.25-4.31[a]



4.20-4.22, 4.32[a]

Compd.	Het	n	Compd.	Het	n
4.25[a]		4	**4.14**		4
4.26[a]		6	**4.15**		5
4.9		7	**4.16**		6
4.27[a]	2-amino-4-methylthiazol-5-yl	8	**4.17**	imidazol-4-yl	7
4.28[a]		10	**4.31**[a]		8
4.29[a]		14	**4.18**		14
4.30[a]		20	**4.19**		20
4.10	2-aminothiazol-5-yl	6	**4.20**	-	4
4.11		8	**4.21**		6
4.12		14	**4.32**[a]		8
4.13		20	**4.22**		20
4.24	1,2,4-triazol-5-yl	8			

[a] Compounds **4.25-4.32** were provided by Dr. A. Kraus. For experimental data see Ref.[29]

All compounds were examined for histamine H_2R agonism on human (h) and guinea pig (gp) H_2 receptors in steady-state GTPase assay using membranes of Sf9 insect cells expressing hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins, respectively (Table 4.2).[30] In addition, selected compounds were investigated at the isolated spontaneously beating gp right atrium[31] as a pharmacological standard model for the characterization of H_2R ligands (positive chronotropic response) (Table 4.3), and in the GTPγS binding assay on gpH_2R-$Gs\alpha_S$ fusion proteins. Furthermore, with respect to information about the molecular determinants of different agonist potencies at human and guinea pig H_2R orthologs, selected bivalent ligands were tested on H_2R mutants, in which Cys-17 and Ala-271 in the hH_2R were replaced by Tyr-17 and Asp-271 as in the gpH_2R and four different amino acids in the e2 loop were reciprocally mutated (hH_2R-C17Y-A271D-$Gs\alpha_S$, hH_2R-C17Y-$Gs\alpha_S$, hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$) (Table 4.4).[30,32-33] Moreover, the histamine receptor selectivities of representative compounds were explored

in GTPase assays using recombinant human histamine H_1, H_3 and H_4 receptors (Table 4.5).

4.3.1 Histamine H_2 receptor agonism

4.3.1.1 H_2R agonism at human and guinea pig H_2R fusion proteins in the GTPase assay

Pharmacophore duplication led to potent partial to full agonists in the GTPase assay at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins (Table 4.2). Investigations of three different series of twin compounds containing either two (2-amino-4-methylthiazolyl)propyl-guanidines (**4.9** and **4.25-4.30**), two (2-aminothiazolyl)propylguanidines (**4.10-4.13**) or two imidazolylpropylguanidines (**4.14-4.19** and **4.31**) revealed the following results (see Figure 4.2): when increasing the spacer length from four to twenty C-atoms, covering a distance of ≈ 6 to ≈ 27 Å between the carbonyl groups, highest potencies were obtained with octanedioyl or decanedioyl spacers at both, hH_2R-$Gs\alpha_S$ (pEC_{50} values ≤ 8.2) and gpH_2R-$Gs\alpha_S$ fusion proteins (pEC_{50} values ≤ 9.4). These compounds exceeded the potency of histamine up to 200 and over 3000 times at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Further extension of the spacer length resulted in a significant drop in potency or in a complete loss of agonistic activity at hH_2R-$Gs\alpha_S$ and switch to H_2R antagonism (pK_B values, **4.13**: 5.8, **4.19**: 6.4, **4.30**: 6.1).

Homobivalent 2-aminothiazoles lacking the 4-methyl substituent showed slightly decreased potencies but increased efficacies compared to their methylated analogs (**4.10** *vs.* **4.26**, **4.11** *vs.* **4.27**, **4.12** *vs.* **4.29**, Fig. 4.2 **C**, **D**) at both, the hH_2R-$Gs\alpha_S$ and the gpH_2R-$Gs\alpha_S$. Compounds **4.10** and **4.11** were full agonists at gpH_2R-$Gs\alpha_S$. Compared to the corresponding 2-amino-4-methylthiazoles, most imidazoles (**4.16-4.18**, **4.31**) were nearly equipotent at hH_2R-$Gs\alpha_S$ and slightly less potent at gpH_2R-$Gs\alpha_S$. Furthermore, the imidazoles revealed the highest efficacies among the three structural classes, resulting in full agonists at gpH_2R-$Gs\alpha_S$.

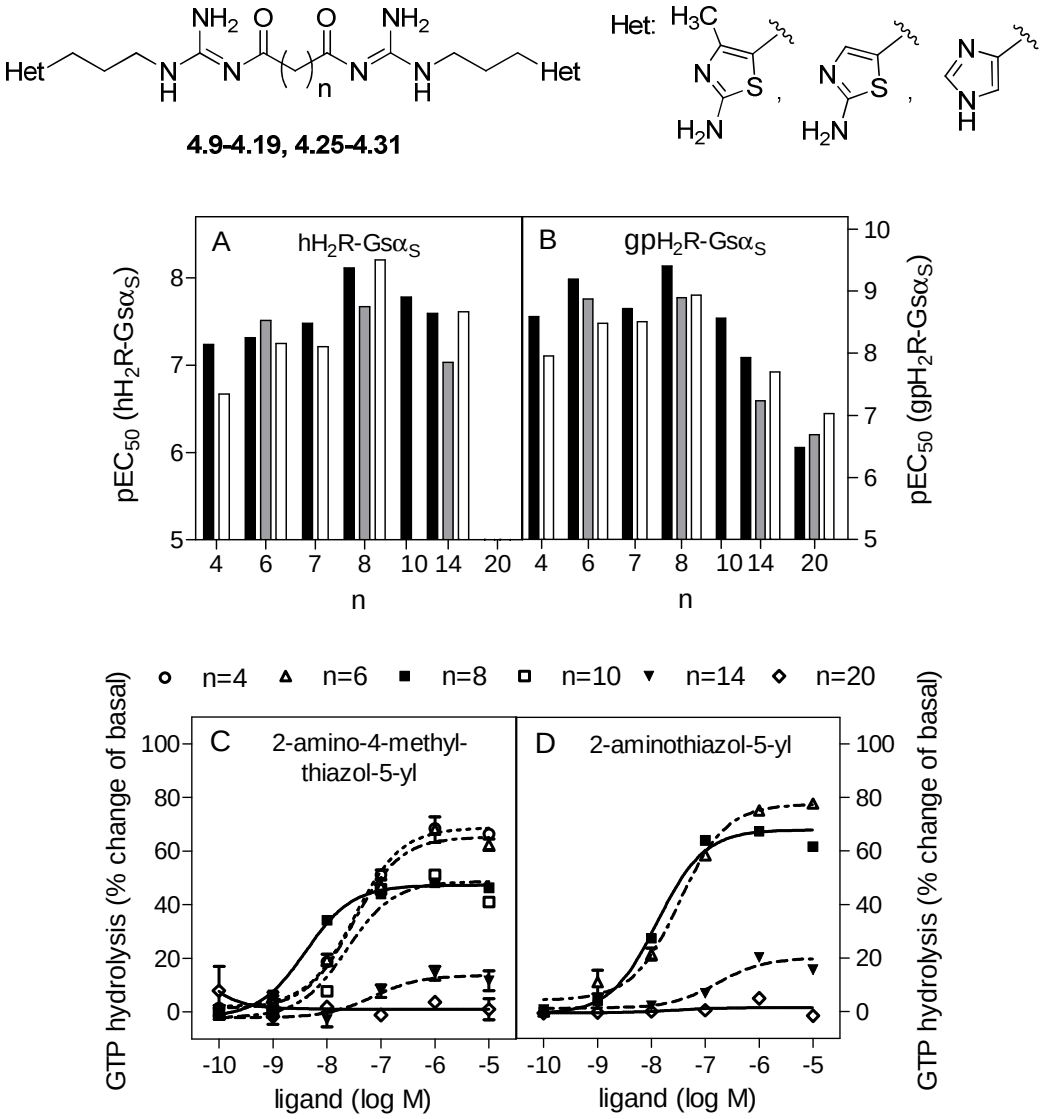


Figure 4.2. Effect of spacer length (n = number of methylene groups) on the potency of symmetrical bivalent acylguanidines. **A**, **B:** Correlation between potencies of bivalent ligands (Het: 2-amino-4-methylthiazol-5-yl (■), 2-aminothiazol-5-yl (■) and imidazol-4-yl (□)) at hH_2R-Gsα_S (**A**) and gpH_2R-Gsα_S (**B**). At hH_2R-Gsα_S, compounds with spacer length of 20 methylene groups showed no agonistic activity. **C**, **D**: Histamine H_2R agonism of bivalent 3-(2-amino-4-methylthiazol-5-yl)propylguanidines (**C**) and 3-(2-aminothiazol-5-yl)propylguanidines (**D**) with increasing spacer lengths at hH_2R-Gsα_S. Data of representative experiments, expressed as percentage change in GTPase activity relative to the maximum effect induced by histamine (100 µM).

Very recently, N^G-acylated 1,2,4-triazolylpropylguanidines were identified as selective H_2R agonists with low to moderate potencies.[28] In contrast to 2-aminothiazoles which have raised suspicion to form toxic metabolites after oxidative cleavage of the ring,[34-36] the triazole ring is considered as relatively stable against oxidation by oxygenases[37] and therefore may be a promising alternative bioisostere of the imidazole ring. Hence, a

bivalent compound bearing two 1,2,4-triazolylpropylguanidine residues connected with a decanedioyl spacer (**4.24**) was prepared to evaluate the H_2R agonistic activity. Unfortunately, compound **4.24** showed up to two orders of magnitude lower potencies compared to the corresponding 2-aminothiazoles **4.27** and **4.11** and the imidazole **4.31** at hH_2R-$Gs\alpha_S$ (pEC_{50} values, **4.24**: 6.82, **4.27**: 8.11, **4.11**: 7.67, **4.31**: 8.21) and gpH_2R-$Gs\alpha_S$ (pEC_{50} values, **4.24**: 7.99, **4.27**: 9.41, **4.11**: 8.30, **4.31**: 8.94), respectively. Thus, the 1,2,4-triazole moiety proved to be inappropriate as a bioisosteric replacement of the imidazole ring in bivalent acylguanidine-type H_2R agonists.

Compounds **4.20**-**4.22** and **4.32** with only one imidazolylpropylguanidine pharmacophore and a free carboxylic group were significantly less potent than the corresponding bivalent ligands, but the orders of potencies were in good agreement. Again, an 8- to 10-membered carbon chain was optimal with respect to H_2R agonistic activity, whereas a 20-membered carbon chain resulted in a total loss of agonistic activity at the hH_2R-$Gs\alpha_S$. However, these compounds can only be considered an approximation to monomeric analogs as the alkyl chain including the carboxylic group, which is converted to an acylguanidine in the twin compounds, may also confer to H_2R binding. To estimate the contribution of the second pharmacophoric moiety, the activities of bivalent ligands should be compared with more appropriate monomeric compounds. This issue has been investigated in chapter 5 in more detail.

Table 4.2. Potencies and efficacies of bivalent acylguanidines and reference compounds at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins in the steady-state GTPase assay.[a]

Compd.	hH_2R-$Gs\alpha_S$			gpH_2R-$Gs\alpha_S$			EC_{50} (hH_2R-$Gs\alpha_S$)/ EC_{50} (gpH_2R-$Gs\alpha_S$)
	E_{max} ± SEM	$pEC_{50}/(pK_B)$ ± SEM	Pot_{rel}	E_{max} ± SEM	pEC_{50} ± SEM	Pot_{rel}	
His[30]	1.00	5.90 ± 0.09	1.0	1.00	5.92 ± 0.09	1.0	1.05
Amt[30]	0.91 ± 0.02	6.72 ± 0.10	6.6	1.04 ± 0.01	6.72 ± 0.09	6.3	1.00
4.25	0.68 ± 0.03	7.24 ± 0.22	21.9	0.90 ± 0.05	8.59 ± 0.30	467.7	22.39
4.26	0.62 ± 0.03	7.32 ± 0.23	26.3	0.81 ± 0.03	9.20 ± 0.16	1,905.5	75.97
4.9	0.48 ± 0.04	7.45 ± 0.14	35,5	0.90 ± 0.06	8.56 ± 0.16	436.5	16.24
4.27	0.53 ± 0.04	8.11 ± 0.25	162.2	0.79 ± 0.07	9.41 ± 0.15	3,090.3	19.90
4.28	0.46 ± 0.04	7.78 ± 0.17	75.9	0.66 ± 0.05	8.57 ± 0.32	446.7	6.17
4.29	0.12 ± 0.02	7.59 ± 0.22	49.0	0.51 ± 0.02	7.93 ± 0.47	102.3	2.19
4.30[29]	(-	(6.11 ± 0.15)	-)[b]	0.58 ± 0.02	6.48 ± 0.37	3.6	-

Table 4.2. (continued)

4.10	0.79 ± 0.03	7.51 ± 0.02	40.7	1.00 ± 0.03	8.87 ± 0.28	891.3	22.89
4.11	0.75 ± 0.03	7.67 ± 0.07	58.9	0.94 ± 0.01	8.30 ± 0.22	239.9	4.27
4.12	0.14 ± 0.01	7.03 ± 0.13	13.5	0.59 ± 0.01	7.23 ± 0.19	20.4	1.58
4.13	(-	(5.77)	-)[b]	0.36 ± 0.01	6.69 ± 0.01	5.9	-
4.14	0.68 ± 0.04	6.67 ± 0.34	5.9	1.00 ± 0.02	7.96 ± 0.07	109.7	19.51
4.15	1.02 ± 0.06	7.24 ± 0.08	21.9	1.16 ± 0.15	8.80 ± 0.09	758.6	36.31
4.16	0.77 ± 0.12	7.25 ± 0.16	22.4	1.18 ± 0.01	8.49 ± 0.33	371.5	17.35
4.17	0.88 ± 0.03	7.21 ± 0.04	20.4	1.19 ± 0.02	8.51 ± 0.30	389.1	19.95
4.31[29]	0.81 ± 0.02	8.21 ± 0.07	204.2	0.98 ± 0.05	8.94 ± 0.16	1,047.1	5.36
4.18	0.29 ± 0.08	7.61 ± 0.18	51.3	0.85 ± 0.10	7.70 ± 0.26	60.4	1.23
4.19	(-	(6.57 ± 0.07)	-)[b]	0.19 ± 0.03	7.46 ± 0.12	34.7	-
4.24	0.49 ± 0.03	6.82 ± 0.05	10.5	0.95 ± 0.04	7.99 ± 0.02	117.5	14.79
4.20	0.49 ± 0.03	5.73 ± 0.01	0.7	0.79 ± 0.02	6.09 ± 0.02	1.5	2.29
4.21	0.79 ± 0.04	6.87 ± 0.07	9.3	0.99 ± 0.01	6.99 ± 0.03	11.8	1.32
4.32[29]	0.67 ± 0.03	7.10 ± 0.07	15.9	0.97 ± 0.04	6.82 ± 0.26	7.4	0.52
4.22	(-	-	-)[b]	0.68 ± 0.0	5.41 ± 0.01	0.3	-

[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 10 μM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (100 μM) ranged between ≈ 2 and 13 $pmol \cdot mg^{-1} \cdot min^{-1}$. The efficacy ($E_{max}$) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of 2-6 independent experiments performed in duplicate. The relative potency of histamine was set to 1.0, and the potencies of other agonists were referred to this value. [b] No agonistic activity.

In agreement with previous studies on monovalent acylguanidine-type H_2R agonists all bivalent compounds exhibited higher potencies and efficacies at gpH_2R-$Gs\alpha_S$ relative to hH_2R-$Gs\alpha_S$ (see Figure 4.3).[1,25,30,38] Interestingly, compounds **4.10**, **4.15**, **4.17** and **4.25-4.27** were 20 to 76 times more potent at the gpH_2R-$Gs\alpha_S$ compared to hH_2R-$Gs\alpha_S$ and therefore exhibited the highest preference for gpH_2R-$Gs\alpha_S$ among acylguanidines known so far. Compounds **4.10**, **4.26, 4.27** and **4.31** (EC_{50} values at gpH_2R-$Gs\alpha_S$: 0.39 nM – 1.35 nM) turned out to be the most potent acylguanidine-type H_2R agonists identified in the GTPase assay.

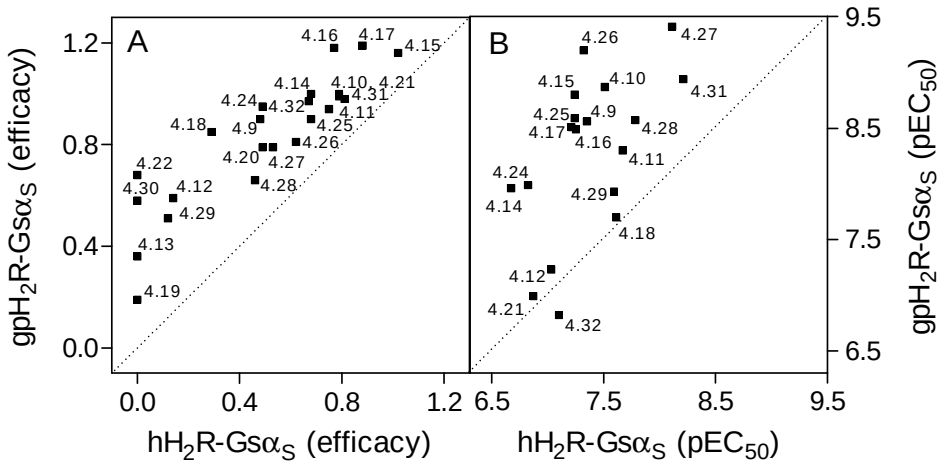
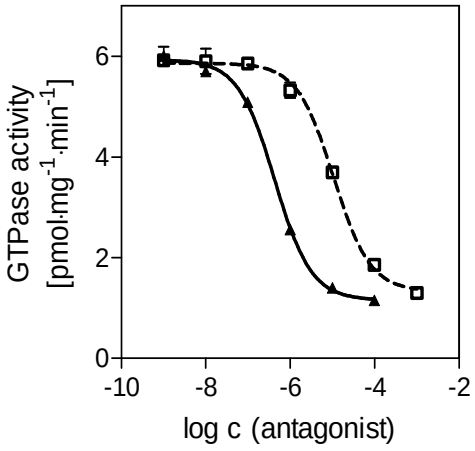


Figure 4.3. Efficacies and potencies of the title compounds at hH_2R-$Gs\alpha_S$ in comparison with gpH_2R-$Gs\alpha_S$ as determined in the steady-state GTPase assay. The dotted lines represent the line of identity. **A**: Plot of efficacies at gpH_2R-$Gs\alpha_S$ *vs.* hH_2R-$Gs\alpha_S$. **B**: Plot of pEC_{50} at gpH_2R-$Gs\alpha_S$ *vs.* hH_2R-$Gs\alpha_S$. In **B**, compounds **4.13**, **4.19**, **4.20**, **4.22** and **4.30** are not demonstrated as they are very weak agonists or antagonists.

Given that the steady-state GTPase assay is an artificial test system using membrane preparations instead of intact cells, G-proteins might be directly accessible to the investigated compounds, i.e. the possibility of receptor-independent G-protein activation has to be taken into account. Direct G-protein activation has been reported for various cationic-amphiphilic compounds including local anesthetics, β-adrenoceptor antagonists and wasp venom mastoparan.[39-42] Direct G-protein activation by histamine receptor ligands was reported to occur at concentrations higher than 10 µM.[40-41,43] To verify the H_2R-mediated effect and to exclude direct G-protein activation as a mechanism of GTPase stimulation, selected bivalent H_2R agonists were investigated in the presence of the H_2R antagonists famotidine and ranitidine in

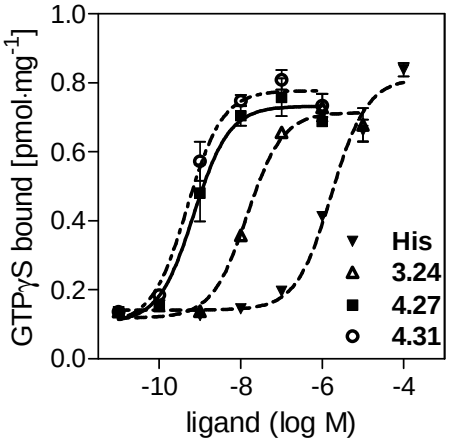


	K_B [nM]
famotidine	154 ± 67
ranitidine	1755 ± 930

Figure 4.4. Concentration-dependent inhibition of GTP hydrolysis by famotidine (solid line) and ranitidine (dashed line) using **4.31** as the H_2R agonist at a concentration of 1 nM at the gpH_2R-$Gs\alpha_S$. Data points shown are means ± SEM of representative experiments performed in duplicate.

the GTPase assay as shown for **4.31** in Figure 4.4. At both, hH_2R-$Gs\alpha_S$ (data not shown) and gpH_2R-$Gs\alpha_S$, the **4.31**-stimulated GTP hydrolysis was inhibited in a concentration-dependent manner, confirming the measured GTPase activity to be H_2R-mediated. The calculated K_B values of famotidine (154 ± 67 nM, Fig. 4.4) and ranitidine (1755 ± 930 nM, Fig. 4.4) determined against **4.31** at gpH_2R-$Gs\alpha_S$, respectively, are in the same range as data obtained from GTPase assays using histamine as the H_2R agonist (reported K_B values, famotidine: 38 ± 3 nM, ranitidine: 1000 ± 170 nM).[44]

For comparison, examples of acylguanidines (**3.24**, **4.27**, **4.31**) were additionally investigated in GTPγS binding assays using membrane preparations of Sf9 cells expressing the gpH_2R-$Gs\alpha_S$ fusion protein (cf. Figure 4.5). The determined pEC_{50} values and intrinsic activities were in good agreement with the data from the GTPase assay.



	pEC_{50}	E_{max}
His	5.79 ± 0.02	1.00
3.24	7.80 ± 0.07	0.92 ± 0.03
4.27	9.01 ± 0.14	0.97 ± 0.04
4.31	9.31 ± 0.01	1.04 ± 0.03

Figure 4.5. Histamine H_2 receptor agonism of representative bivalent ligands **4.27** (■) and **4.31** (○) compared to the monovalent ligand **3.24** (Δ) and histamine (▼) in the GTPγS binding assay using membranes expressing gpH_2R-$Gs\alpha_S$ fusion proteins. Data points are means of representative experiments performed in duplicate, analyzed by nonlinear regression for best fit to sigmoidal concentration-response curves.

4.3.1.2 H_2R agonism on the isolated guinea pig right atrium

In addition to the studies on membrane preparations, representative bivalent H_2R agonists were investigated on the isolated spontaneously beating gp right atrium as a more complex, well established standard model for the characterization of H_2R ligands. The obtained data (Table 4.3) were in good agreement with the results from the GTPase assays on the gpH_2R-$G_{s\alpha S}$ fusion proteins in terms of both potencies and intrinsic activities. The structure-activity relationships and the orders of potencies derived from the

guinea pig right atrium were comparable to those derived from the GTPase assay. However, the agonist potency of the long chain members of the series (**19**, **20** and **21**) decreased substantially in the organ assay compared with the GTPase assay. The combination of two hetarylpropylguanidine moieties with octanedioyl, nonanedioyl or decanedioyl spacers (pEC_{50} values, **4.9**: 9.08, **4.26**: 9.61, **4.27**: 8.93, **4.31**: 9.22) led to the most potent agonists at the gp right atrium known so far, surpassing up to 4000 times the potency of histamine in increasing heart rate. In addition, similar to the results from the GTPase assay the exchange of 2-amino-4-methylthiazole against imidazole rings (**4.27** *vs.* **4.31**) increased the efficacy at the gpH_2R (0.62 → 0.91). In agreement with monovalent acylguanidines (see chapter 3), the positive chronotropic response was sensitive against the H_2R antagonist cimetidine (10-100µM), thus, confirming a H_2R-mediated effect of bivalent acylguanidines (data not shown).

Table 4.3. Histamine H_2 receptor agonism at the spontaneously beating guinea pig right atrium.

Compd.	pEC_{50} ± SEM[a]	Pot_{rel}[b]	E_{max} ± SEM[c]
His	6.00 ± 0.10	1.0	1.0
Amt[45]	6.21 ± 0.09	1.62	0.95 ± 0.02
4.9	9.08 ± 0.05	1,210.1	0.71 ± 0.05
4.25	8.59 ± 0.07	389.0	0.88 ± 0.03
4.26	9.61 ± 0.03	4,070.0	0.64 ± 0.03
4.27	8.93 ± 0.14	847.0	0.62 ± 0.04
4.29	6.26 ± 0.14	1.82	0.53 ± 0.11
4.30	5.10 ± 0.13	0.13	0.62 ± 0.07
4.31	9.22 ± 0.06	1,640.0	0.91 ± 0.04

[a] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$; summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, N = 16); data shown are means ± SEM of three to five experiments; [b] Potency relative to histamine = 1.0; [c] Intrinsic activity, maximal response relative to the maximal increase in heart rate induced by the reference compound histamine = 1.0.

4.3.1.3 Interaction with the recognition site of H_2R dimers or binding to different sites of one protomer?

The structure-activity relationships of bivalent H_2R agonistic acylguanidines, resulting from GTPase, GTPγS binding and guinea pig right atrium assays, are not compatible with the possible role of such ligands as compounds "bridging" the recognition (orthosteric) sites of receptor dimers. The spacers of the highly potent agonists **4.9**-**4.11, 4.16, 4.26,**

4.27 and **4.31** are too short to simultaneously occupy two H_2R protomers. The presumed optimal spacer length of ≈ 22-27 Å may be attained only by compounds **4.13**, **4.19** and **4.30** (n = 20, carbonyl-carbonyl distance 26.4 Å with fully extended chain). However, spacers with 14 and 20 carbon atoms resulted in weak agonism (gpH_2R-$Gs\alpha_S$) or loss of agonistic activity and conversion to antagonism (hH_2R-$Gs\alpha_S$). Thus, the remarkable increase in potency compared to monovalent H_2R agonists is presumably due to interaction with an accessory (allosteric?) binding site at the same receptor molecule rather than to occupation of two protomers of a receptor dimer. In fact, many bivalent GPCR ligands with drastically increased activities relative to the monovalent parent compounds in spite of insufficient linker lengths for bridging of receptor protomers have been reported.[24,46-47] The differences in potencies and intrinsic activities between the data obtained on human and guinea pig H_2R orthologs may be interpreted as a hint to species-dependent molecular determinants possibly affecting both the orthosteric and the putative accessory binding site. Therefore, additional studies on H_2R mutants were performed.

4.3.2 Agonistic activity on histamine H_2R mutants/chimera

Unlike small H_2R agonists such as histamine and amthamine, which are full agonists at human and guinea pig H_2Rs, all bulky guanidine-type H_2R agonists turned out to be significantly more potent and efficacious at the gpH_2R relative to the hH_2R.[1,25,30,38] This species-selective activation is also true for bivalent compounds as revealed in the GTPase assay (cf. Table 4.2 and Figure 4.3). These differences may result from species-dependent interactions with both the orthosteric and the putative accessory binding site. The latter probably resides in the extracellular domains, and amino acids in the e2 loop are possible candidates to interact with bivalent ligands. Based on the crystal structure of rhodopsin,[48] the participation of residues of the e2 loop to the binding pocket was proposed and already experimentally demonstrated for some members of class A GPCRs.[49-52] However, the very recently resolved crystal structures of the turkey β_1- and the human β_2-adrenergic receptor indicate a certain contribution of a phenylalanine in the e2 loop to agonist and antagonist binding,[53-54] but this residue belongs to the orthosteric site. Since the e2 loops of the hH_2R and the gpH_2R differ by only four amino acids outside the orthosteric binding pocket (hH_2R: G167, H169, T171, S172 *vs.* gpH_2R: D167, D169, I171, V172), reciprocal mutation (hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$) is an approach to probe whether species selectivity of bivalent ligands depends on an accessory function of the e2 loop.

Application of this approach to *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated analogs indicated that the e2 loop does not contribute to species-selectivity of monovalent H_2R agonists.[33] Investigations of selected bivalent acylguanidines on the reciprocal mutants led to rather ambivalent results. As summarized in Table 4.4, all investigated compounds exhibited similar potencies and efficacies at mutant hH_2R-gpE2-$Gs\alpha_S$ and wild-type hH_2R-$Gs\alpha_S$. At mutant gpH_2R-hE2-$Gs\alpha_S$ the compounds are equi-efficacious compared to the wild-type gpH_2R-$Gs\alpha_S$. However, the pEC_{50} values are significantly reduced by 0.5 to 0.9 in the case of 2-amino-4-methylthiazolyl compounds (**4.26**, **4.27**) except **4.29**, whereas the potencies of imidazolyl (**4.31**) and 2-aminothiazolyl derivatives (**4.10**, **4.11**) remain nearly unchanged (Figure 4.6 **A**). Hence, these results do not indicate direct interactions of the mutated residues with the bivalent ligands. However, the integrity of the e2 loop seems to be necessary for high-affinity gpH_2R binding of bivalent 2-amino-4-methylthiazoles. It is not obvious whether the detrimental effect of the mutations is directly based on the modification of an accessory site in the extracellular region or indirectly due to conformational changes of the orthosteric site.

Furthermore, as predicted by H_2R models and verified by site-directed mutagenesis studies, the preference of the guanidine-type agonists for the gpH_2R is strongly dependent on two amino acids, Tyr-17 and Asp-271 in TM 1 and TM 7, respectively, which are thought to stabilize an active receptor conformation via direct or through-water interactions.[30,32] Cys-17 and Ala-271 in the hH_2R cannot fulfill this function. Investigations of selected bivalent acylguanidines on H_2R mutants (Table 4.4, Figure 4.6 **B**), in which Cys-17 and Ala-271 of the hH_2R were replaced by the corresponding amino acids Tyr-17 and Asp-271 of the gpH_2R (hH_2R-C17Y-A271D-$Gs\alpha_S$, hH_2R-C17Y-$Gs\alpha_S$) confirmed that both Tyr-17 in TM1 and Asp-271 in TM7 or at least Asp-271 are key residues for highly potent and efficacious H_2R activation. The sensitivity of the hH_2R-C17Y-A271D-$Gs\alpha_S$ double mutant against agonist stimulation was shifted to that of the gpH_2R isoform. The single Cys-17-Tyr mutation had only slight or in some cases even detrimental effects on hH_2R potency and efficacy.

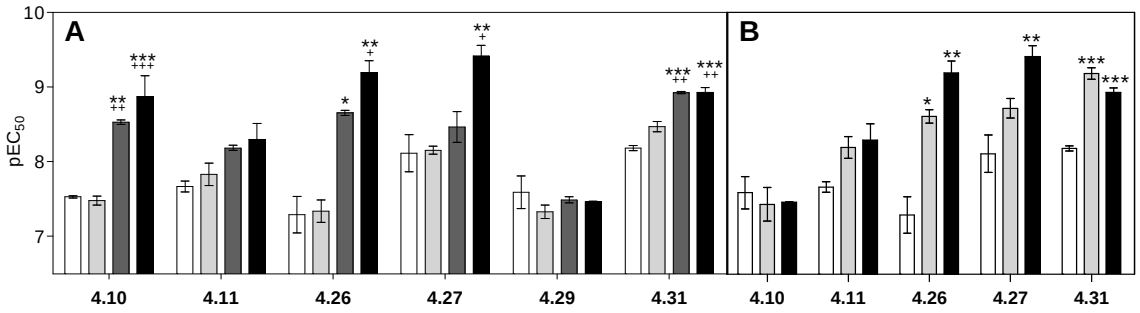


Figure 4.6. Comparison of the agonistic potencies of selected bivalent ligands at wild-type and mutant human and guinea pig H_2 receptors as determined in GTPase assays. Data shown are the means ± SEM of two to five independent experiments performed in duplicate. pEC_{50} values were compared with each other using one-way ANOVA, followed by Bonferroni`s multiple comparison test. **A**: pEC_{50} values of **4.10**, **4.11**, **4.26**, **4.27**, **4.29** and **4.31** at hH_2R-$Gs\alpha_S$ (□) *vs.* hH_2R-gpE2-$Gs\alpha_S$ (■) *vs.* gpH_2R-hE2-$Gs\alpha_S$ (■) *vs.* gpH_2R-$Gs\alpha_S$ (■) fusion proteins. pEC_{50} significantly different to: *hH_2R-$Gs\alpha_S$, $^{+}hH_2R$-gpE2-$Gs\alpha_S$ or °gpH_2R-hE2-$Gs\alpha_S$; one symbol: $p < 0.05$, two symbols: $p < 0.01$, three symbols: $p < 0.001$; 95% confidence interval. **B**: pEC_{50} values of **4.10**, **4.11**, **4.26**, **4.27** and **4.31** at hH_2R-$Gs\alpha_S$ (□) *vs.* hH_2R-C17Y-A271D-$Gs\alpha_S$ double mutant (■) *vs.* gpH_2R-$Gs\alpha_S$ (■). *pEC_{50} significantly different to hH_2R-$Gs\alpha_S$; one symbol: $p < 0.05$, two symbols: $p < 0.01$, three symbols: $p < 0.001$; 95% confidence interval.

Table 4.4. Potencies and efficacies of bivalent acylguanidine-type H_2R agonists at hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$, hH_2R-C17Y-A271D-$Gs\alpha_S$ and hH_2R-C17Y-$Gs\alpha_S$ expressed in Sf9 cell membranes.[a]

Compd.	hH_2R-gpE2-$Gs\alpha_S$		gpH_2R-hE2-$Gs\alpha_S$		hH_2R-C17Y-A271D-$Gs\alpha_S$		hH_2R-C17Y-$Gs\alpha_S$	
	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM
His$^{32-33}$	1.00	6.17 ± 0.07	1.00	5.86 ± 0.05	1.00	6.50 ± 0.01	1.00	6.61 ± 0.11
Amt$^{32-33}$	0.94 ± 0.05	6.86 ± 0.06	0.94 ± 0.06	6.53 ± 0.09	0.97 ± 0.01	7.19 ± 0.02	0.86 ± 0.19	6.93 ± 0.04
4.10	0.69 ± 0.03	7.48 ± 0.06	0.97 ± 0.03	8.53 ± 0.03	nd[b]		0.82 ± 0.12	7.23 ± 0.10
4.11	0.74 ± 0.05	7.83 ± 0.15	0.99 ± 0.05	8.19 ± 0.04	0.79 ± 0.02	8.20 ± 0.14	0.52 ± 0.08	8.05 ± 0.22
4.26[29]	0.65 ± 0.05	7.34 ± 0.15	1.00 ± 0.01	8.65 ± 0.04	0.83 ± 0.02	8.61 ± 0.09	0.33 ± 0.03	8.02 ± 0.08
4.27[29]	0.65 ± 0.01	8.16 ± 0.06	0.86 ± 0.03	8.47 ± 0.21	0.78 ± 0.01	8.72 ± 0.13	0.29 ± 0.02	7.60 ± 0.03
4.29[29]	0.22 ± 0.03	7.33 ± 0.09	0.49 ± 0.01	7.49 ± 0.04	0.17 ± 0.01	7.44 ± 0.23	(-	-)[c]
4.31[29]	0.82 ± 0.01	8.47 ± 0.07	1.11 ± 0.04	8.92 ± 0.01	0.97 ± 0.07	9.19 ± 0.08	0.94 ± 0.06	8.16 ± 0.05

[a] Steady state GTPase activity in Sf9 membranes expressing hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$, hH_2R-C17Y-A271D-$Gs\alpha_S$ and hH_2R-C17Y-$Gs\alpha_S$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 100 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to

sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 1.5 pmol·mg^{-1}·min^{-1} for hH_2R-gpE2-Gsα_S and gpH_2R-hE2-Gsα_S, ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-A271D-Gsα_S and ≈ 0.7 and 1.3 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-Gsα_S. Activities stimulated by histamine (100 μM) ranged between ≈ 2.8 and 5.0 pmol·mg^{-1}·min^{-1} for hH_2R-gpE2-Gsα_S and gpH_2R-hE2-Gsα_S, ≈ 1.1 and 4.5 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-A271D-Gsα_S and ≈ 1.1 and 1.8 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-Gsα_S. The intrinsic activity (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of one to three experiments performed in duplicate. [b] nd: not determined. [c] No agonistic activity.

4.3.3 Receptor selectivity

To determine the histamine receptor selectivity profile (human H_2R *vs.* H_1R, H_3R, H_4R), representative compounds were investigated in GTPase assays on recombinant human H_1, H_3 and H_4 receptors for agonism and antagonism, respectively (Table 4.5). These experiments were performed at membranes of Sf9 insect cells co-expressing the hH_1R plus RGS4, co-expressing the hH_3R plus Gα_{i2} plus G$\beta_1\gamma_2$ plus RGS4 or co-expressing the hH_4R-RGS19 fusion protein plus Gα_{i2} plus G$\beta_1\gamma_2$. Recently reported monovalent N^G-acylated aminothiazolylpropylguanidine-type H_2R agonists proved to be devoid of agonistic and antagonistic activities or to have only negligible effects on histamine receptors other than the H_2R (see chapter 3).[1] This also holds for bivalent ligands: the investigated compounds containing two 2-aminothiazole moieties (**4.10**, **4.11** and **4.25-4.29**) showed only very weak antagonistic effects on H_1, H_3 and H_4 histamine receptors. By contrast, compounds containing two imidazole rings (**4.14-4.19** and **4.31**) showed, in addition to H_2R agonism, significant agonistic, antagonistic or inverse agonistic activities at the other histamine receptor subtypes, depending on the spacer length. In particular, the imidazolylpropylguanidines with octane- (**4.16**) and decanedioyl spacer (**4.31**) turned out to be highly potent hH_3R and hH_4R partial agonists in the low nanomolar range and therefore may be promising starting points for the development of highly potent H_3R and H_4R agonists. Hence, the replacement of the privileged imidazolylpropylguanidine portion with an aminothiazolylpropylguanidine moiety strongly favors the selectivity for the H_2R in the case of both monovalent and bivalent N^G-acylated guanidines. Again, these data confirm the working hypothesis that the 2-aminothiazole and the imidazole moiety are bioisosteric groups at the H_2R but not at the H_3R and the H_4R.

Table 4.5. Histamine receptor subtype selectivity of selected bivalent ligands. Agonistic, antagonistic and inverse agonistic effects of bivalent ligands at hH_1R + RGS4, hH_2R-$Gs\alpha_S$, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ + RGS4 and hH_4R-RGS19 + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ expressed in Sf9 cell membranes.[a]

Compd.	hH_1R	hH_2R		hH_3R		hH_4R	
	(pK_B)	**pEC_{50} (pK_B)**	**E_{max}**	**pEC_{50} (pK_B)**	**E_{max}**	**pEC_{50} (pK_B)**	**E_{max}**
4.25	(< 6.00)	7.24 ± 0.22	0.68 ± 0.03	(< 5.00)	-	(< 6.00)	-
4.26	(< 6.00)	7.32 ± 0.23	0.62 ± 0.03	(< 5.00)	-	(< 6.00)	-
4.27	(6.01 ± 0.07)	8.11 ± 0.25	0.53 ± 0.04	(< 5.00)	-	(< 6.00)	-
4.29	(< 6.00)	7.59 ± 0.22	0.12 ± 0.02	(< 6.00)	-	(< 6.00)	-
4.10	(< 6.00)	7.51 ± 0.02	0.79 ± 0.03	(6.36 ± 0.11)	-	(< 6.00)	-
4.11	(< 6.00)	7.67 ± 0.07	0.75 ± 0.03	(< 5.00)	-	(< 6.00)	-
4.14	(6.13 ± 0.22)	6.67 ± 0.34	0.68 ± 0.04	< 5.00	– 0.22 ± 0.03	7.10 ± 0.12	0.42 ± 0.01
4.16	(6.70 ± 0.07)	7.25 ± 0.16	0.77 ± 0.12	8.38 ± 0.11	0.37 ± 0.08	7.38 ± 0.02	0.51 ± 0.04
4.31	(6.32 ± 0.16)	8.21 ± 0.07	0.81 ± 0.02	8.75 ± 0.06	0.63 ± 0.08	8.07 ± 0.19	0.44 ± 0.05
4.18	(< 6.00)	7.61 ± 0.18	0.29 ± 0.08	< 6.00	– 1.02 ± 0.02	6.47 ± 0.04	– 0.29 ± 0.09
4.19	(< 6.00)	(6.57 ± 0.07)	-	6.35 ± 0.03	– 0.77 ± 0.02	< 6.00	– 0.86 ± 0.02

[a] Steady state GTPase activity in Sf9 membranes expressing hH_1R+RGS4, hH_2R-$Gs\alpha_S$, hH_3R+$G\alpha_{i2}$+$G\beta_1\gamma_2$+RGS4 and hH_4R-RGS19+$G\alpha_{i2}$+$G\beta_1\gamma_2$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 100 µM as appropriate to generate saturated concentration-response curves. For antagonism, reaction mixtures contained histamine (hH_1R: 1 µM; hH_3R, hH_4R: 100 nM) and ligands at concentrations from 1 nM to 1 mM. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 1.5 and 2.5 $pmol \cdot mg^{-1} \cdot min^{-1}$, and activities stimulated by histamine (10 µM) ranged between ≈ 3.5 and 4.5 $pmol \cdot mg^{-1} \cdot min^{-1}$. Data shown are mean values from one to four experiments performed in duplicate. IC_{50} values were converted to K_B values using the Cheng-Prusoff equation.[55] Efficacy (E_{max}) relative to the maximal response of histamine = 1.00. Negative values refer to inverse agonistic effects. [b] For general structure of bivalent hetarylpropylguanidines see Table 4.1.

4.4 Summary

Starting from N^G-acylated hetarylpropylguanidines which were recently discovered in our laboratory as a new class of potent H_2R agonists[1,25] several bivalent histamine H_2R agonists were synthesized by connecting two hetarylpropylguanidine entities by N^G-acylation with alkanedioic acids of various chain lengths (6 – 27 Å). The pharmacophore duplication resulted in novel hH_2R and gpH_2R agonists which may serve as pharmacological tools for more detailed investigations of the H_2R. The bivalent ligands proved to be partial to full H_2R agonists, up to two orders of magnitude more potent than monovalent acylguanidines and up to 4000 times more potent than histamine at the gpH_2R (compounds with octanedioyl to decanedioyl spacers). These are the most potent histamine H_2R agonists known to date. However, the results of this study, in particular the structure-activity relationships with respect to spacer length, do not support the hypothesis of simultaneous occupation of the recognition sites of neighboring protomers. The spacer optimum rather suggests that the remarkable increase in potency compared to monovalent H_2R agonists is due to the interaction with an accessory (allosteric?) binding site at the same receptor molecule. Investigations on hH_2R and gpH_2R mutants, aiming at identifying molecular determinants of the putative accessory binding site, confirmed the key role of non-conserved Tyr-17 and Asp-271 in TM1 and TM7 in the gpH_2R for species-selective H_2R activation and suggested that the e2 loop does not participate in direct ligand - receptor interaction. In order to further elaborate structure-activity relationships with respect to the role and the interaction site of the spacer and the second set of pharmacophoric groups, it is necessary to synthesize and pharmacologically characterize additional compounds with different spacers, e.g. more bulky, rigid and/or hydrophilic spacers, and distinct pharmacophores as well as non H_2R-specific moieties.

4.5 Experimental section

4.5.1 Chemistry

4.5.1.1 General conditions

See section 3.5.1.1

4.5.1.2 Preparation of the N^G-Boc-protected building block 4.8

(E)-Methyl 3-(1*H*-imidazol-4-yl)propenoate (4.1)[27]

To a solution of urocanic acid (10 g, 72.4 mmol) and anhydrous Na_2SO_4 (1.5 g) in 100 ml MeOH/abs was added 6 ml of conc. H_2SO_4. After refluxing for 30 h, the solvent was removed under reduced pressure. The residue was dissolved in a small amount of water, neutralized with saturated $NaHCO_3$/aq and extracted three times with EtOAc. After drying over $MgSO_4$, the solvent was evaporated *in vacuo* to give **4.1** in 94 % yield (10.5 g) as white solid. ^{1}H-NMR (CD_3OD) δ (ppm): 6.33 (s, 1H, Im-2-***H***), 6.18 (d, ^3J = 15.9 Hz, 1H, Im-4-CHC***H***), 2.37 (s, 3H, OC$\boldsymbol{H}_3$); EI-MS (70 eV) m/z (%): 152 ($M^{+\bullet}$, 50); $C_7H_8N_2O_2$ (152.15).

Methyl 3-(1*H*-imidazol-4-yl)propanoate (4.2)[56]

To a solution of **4.1** (9.9 g, 65.1 mmol) in 120 ml of MeOH was added 1.0 g of Pd/C (10 %) at room temperature under stirring. The mixture was hydrogenated at 5 bar for 24 h. After completion of reaction (TLC control) the mixture was filtered through a Celite pad, which was rinsed with MeOH, and the solution was concentrated *in vacuo* to get **4.2** (10 g, 100 %) as white solid. mp 107-109 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.51 (s, 1H, Im-2-***H***), 6.75 (s, 1H, Im-5-***H***), 3.59 (s, 3H, OC$\boldsymbol{H}_3$), 2.75 (t, ^3J = 7.4 Hz, 2H, Im-4-C$\boldsymbol{H}_2$), 2.59 (t, ^3J = 7.3 Hz, 2H, Im-4-CH_2C$\boldsymbol{H}_2$); EI-MS (70 eV) m/z (%): 154 ($M^{+\bullet}$, 35); $C_7H_{10}N_2O_2$ (154.17).

Methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate (4.3)[57]

To a suspension of **4.2** (9.2 g, 48.3 mmol) and NEt_3 (19 ml, 136 mmol) in 120 ml MeCN was added dropwise a solution of trityl chloride (15 g, 54 mmol) in 120 ml MeCN under external ice-cooling. After the addition was completed, the mixture was allowed to warm to room temperature and stirring was continued for 12 h. After removing the solvent under reduced pressure, the resulting solid was suspended in 300 ml H_2O and stirred for 1 h. The solid was filtrated and recrystallized from dry EtOH yielding **4.3** (15.1 g, 79 %) as white solid. mp 131 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.07 (d, ^4J = 1.6 Hz, 1H, Im-2-***H***), 7.41-7.07 (m, 15H, CPh_3), 6.77 (d, ^4J = 1.5 Hz, 1H, Im-5-***H***), 3.62 (s, 3H, OC$\boldsymbol{H}_3$), 3.08 (t, ^3J = 7.0 Hz, 2H, Im-4-C$\boldsymbol{H}_2$), 2.87 (t, ^3J = 7.0 Hz, 2H, Im-4-CH_2C$\boldsymbol{H}_2$); EI-MS (70eV) m/z (%): 396 ($M^{+\bullet}$, 10); $C_{26}H_{24}N_2O_2$ (396.24).

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol (4.4)[57]

To a suspension of $LiAlH_4$ (1.9 g, 50 mmol) in 75 ml freshly distilled THF and 25 ml Et_2O/abs was added **4.3** (10.0 g, 25 mmol) in portions under argon atmosphere and cooling with ice. After the addition was completed, the mixture was allowed to warm to room temperature and refluxed for 2 h. The excess $LiAlH_4$ was decomposed by dropwise addition of 0.1 N NaOH. The solution was extracted several times with DCM, dried over Mg_2SO_4 and the solvent removed in *vacuo*. The residue was purified by flash chromatography ($CHCl_3$/MeOH 95/5 v/v) to obtain **4.4** (6.9 g, 74 %) as white solid. mp 138 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.76 (d, ^4J = 1.4 Hz, 1H, Im-2-***H***), 7.34-7.10 (m, 15H, CPh_3), 6.65 (d, ^4J = 1.5 Hz, 1H, Im-5-***H***), 3.71 (t, ^3J = 5.7 Hz, 2H, C***H***$_2$OH), 2.80 (t, ^3J = 6.9 Hz, 2H, Im-4-C***H***$_2$), 1.90 (m, 2H, Im-4-CH$_2$C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 369 (MH^+, 60); $C_{25}H_{24}N_2O$ (368.24).

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-amine (4.6)[29]

4.5 (3.6 g, 10 mmol), phthalimide (1.4 g, 10 mmol) and PPh_3 (2.5 g, 10 mmol) were suspended in 100 ml THF/abs and cooled to 0 °C. DIAD (1 eq) was slowly added drop by drop. After complete addition of DIAD, the mixture was allowed to warm to room temperature and stirred for 24 h. The solvent was removed under reduced pressure and the crude product suspended in 60 ml EtOH. Then, hydrazine hydrate (2.4 ml, 5 mmol) was added and the mixture was refluxed for 1 h. After cooling to room temperature, the precipitate was filtered off and the solvent evaporated *in vacuo*. The residue was subjected to flash chromatography ($CHCl_3$/MeOH/NEt_3 95/4/1 v/v/v) to obtain **4.6** (2.1 g, 58 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.32-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.52 (d, ^4J = 1.2 Hz, 1H, Im-5-***H***), 2.74 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH$_2$), 2.59 (t, ^3J = 7.4 Hz, 2H, Im-4-C***H***$_2$), 1.78 (m, 2H, Im-4-CH$_2$C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 368 (MH^+, 100); $C_{25}H_{25}N_3$ (367.48).

***tert*-Butyl amino(benzyloxycarbonyl(3-(1-trityl-1H-imidazol-4-yl)propyl)amino)-methylenecarbamate (4.7)**[29]

The title compound was prepared according to literature and by analogy with the procedure described for **3.17** and **3.18** (cf. chapter 3).[1] To a suspension of **4.6** (1 eq), **3.3** (1 eq) and $HgCl_2$ (2 eq) in DCM/abs was added NEt_3 (3 eq) and stirred at ambient temperature for 48 h. Subsequently, EtOAc was added and the precipitate filtered over Celite. The crude product was purified by flash chromatography (PE/EtOAc 80/20 v/v) to give the Boc- and Cbz-protected guanidine **4.7** as colorless foam-like solid in almost 100

% yield. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.47 (d, ^4J = 1.2 Hz, 1H, Im-2-***H***), 7.34-7.10 (m, 20H, CPh_3, Ar-***H***), 6.58 (s, 1H, Im-5-***H***), 5.11 (s, 2H, C***H***$_2$Ar), 3.40 (m, 2H, C***H***$_2$NH), 2.60 (t, ^3J = 7.6 Hz, 2H, Im-4-C***H***$_2$), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 1.45 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 644 (MH^+, 100); $C_{39}H_{41}N_5O_4$ (643.77).

***tert*-Butyl amino(3-(1-trityl-1*H*-imidazol-4-yl)propylamino)methylenecarbamate (4.8)**[29]

The title compound was prepared from **4.7** (1.5 g, 2.33 mmol) by hydrogenation over 1 g Pd/C (10 %) in a mixture of 60 ml THF/MeOH (1:1) for 8 days at 8 bar (TLC control). After filtration over Celite and washing with MeOH, the solvent was removed *in vacuo* to yield **4.8** (1.05 g, 88 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.34-7.10 (m, 16H, Im-2-***H***, CPh_3), 6.57 (s, 1H, Im-5-***H***), 3.41 (m, 2H, C***H***$_2$NH), 2.56 (m, 2H, Im-4-C***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$), 1.46 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 510 (MH^+, 100); $C_{31}H_{35}NO_2$ (509.64).

4.5.1.3 Preparation of the N^G-Boc-protected bivalent acylguanidines 4.9a-4.19a and 4.24a

General procedure for the synthesis of Boc-protected bivalent acylguanidines 4.9a-4.19a

DIEA (1 eq) was added to a solution of pertinent dicarboxylic acid (0.5 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs under argon and stirred for 15 min. A solution of **3.17, 3.18** or **4.8** (1 eq) in DCM/abs was added and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed *in vacuo*. The crude product was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) unless otherwise indicated.

***N*^{1},*N*^{9}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)nonanediamide (4.9a)**

The title compound was prepared from azelaic acid (94 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **3.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.9a** (270 mg, 56 %) as yellow-brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm):

3.45 (m, 4H, C***H***$_2$NH), 2.71 (t, ^3J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.31 (m, 4H, COC***H***$_2$), 2.20 (s, 6H, Thiaz-4-C***H***$_3$), 1.88 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.53 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.35 (m, 6H, (C***H***$_2$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 980 (MH^+, 100); $C_{45}H_{74}N_{10}O_{10}S_2$ (979.3).

N^1,N^8-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)aminothiazol-5-yl]-propylamino}methylene)octanediamide (4.10a)

The title compound was prepared from octanedioic acid (70 mg, 0.4 mmol), EDAC (153 mg, 0.8 mmol), HOBt-monohydrate (123 mg, 0.8 mmol), DIEA (0.14 ml, 0.8 mmol) in 5 ml DCM/abs and **3.18** (320 mg, 0.8 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.10a** (170 mg, 45 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.14 Hz, 4H, C***H***$_2$NH), 2.77 (t, ^3J = 7.14 Hz, 4H, Thiaz-5-C***H***$_2$), 2.48 (t, ^3J = 7.41 Hz, 4H, COC***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.67 (m, 4H, COCH$_2$C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.47 (s, 18H, C(C***H***$_3$)$_3$), 1.39 (m, 4H, (C***H***$_2$)$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 937.5 (MH^+, 100); $C_{42}H_{68}N_{10}O_{10}S_2$ (936.46).

N^1,N^{10}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)aminothiazol-5-yl]propylamino}methylene)decanediamide (4.11a)

The title compound was prepared from decanedioic acid (50 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.08 ml, 0.5 mmol) in 5 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.11a** (200 mg, 54 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.04 (s, 2H, Thiaz-4-***H***), 3.48 (m, 4H, C***H***$_2$NH), 2.79 (m, 4H, Thiaz-5-C***H***$_2$), 2.34 (m, 4H, COC***H***$_2$), 1.93 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.56 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.32 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 965.5 (MH^+, 100); $C_{44}H_{72}N_{10}O_{10}S_2$ (964.5).

N^1,N^{16}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)aminothiazol-5-yl]propylamino}methylene)hexadecanediamide (4.12a)

The title compound was prepared from hexadecanedioic acid (70 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.08 ml, 0.5 mmol) in 5 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 3 ml DCM/abs according to the general procedure yielding **4.12a** (160 mg, 62 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.06 (s, 2H, Thiaz-4-***H***), 3.48 (m, 4H, C***H***$_2$NH), 2.81 (t, ^3J = 7.14 Hz, 4H, Thiaz-5-C***H***$_2$), 2.38 (t, ^3J = 6.9 Hz, 4H, COC***H***$_2$), 1.93 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$),

1.57 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.35-1.29 (m, 20H, (C***H***$_2$)$_{10}$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1049.7 (MH^+, 100); $C_{50}H_{84}N_{10}O_{10}S_2$ (1048.58).

N^1,N^{22}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)aminothiazol-5-yl]propylamino}methylene)docosanediamide (4.13a)

The title compound was prepared from docosanedioic acid (77 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.08 ml, 0.5 mmol) in 5 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.13a** (230 mg, 80 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.06 (s, 2H, Thiaz-4-***H***), 3.48 (m, 4H, C***H***$_2$NH), 2.80 (m, 4H, Thiaz-5-C***H***$_2$), 2.39 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 1.93 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.57 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.35-1.24 (m, 32H, (C***H***$_2$)$_{16}$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1133.7 (MH^+, 100); $C_{56}H_{96}N_{10}O_{10}S_2$ (1132.68).

N^1,N^6-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}hexanediamide (4.14a)

The title compound was prepared from hexanedioic acid (60 mg, 0.4 mmol), EDAC (150 mg, 0.8 mmol), HOBt-monohydrate (110 mg, 0.8 mmol), DIEA (0.14 ml, 0.8 mmol) in 5 ml DCM/abs and **4.8** (420 mg, 0.8 mmol) in 3 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **4.14a** (150 mg, 42 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.33-7.12 (m, 32H, Im-2-***H***, CPh_3), 6.54 (m, 2H, Im-5-***H***), 3.47 (m, 4H, C***H***$_2$NH), 2.60 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.34 (m, 4H, COC***H***$_2$), 1.90 (m, 4H, Im-4-CH_2C***H***$_2$), 1.63 (m, 4H, (C***H***$_2$)$_2$), 1.51 (s, 18H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1129 (MH^+, 100); $C_{68}H_{76}N_{10}O_6$ (1129.39).

N^1,N^7-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}heptanediamide (4.15a)

The title compound was prepared from heptanedioic acid (56 mg, 0.35 mmol), EDAC (135 mg, 0.7 mmol), HOBt-monohydrate (107 mg, 0.7 mmol), DIEA (0.12 ml, 0.7 mmol) in 5 ml DCM/abs and **4.8** (360 mg, 0.7 mmol) in 3 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **4.15a** (300 mg, 75 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.36-7.16 (m, 32H, Im-2-***H***, CPh_3), 6.36 (m, 2H, Im-5-***H***), 3.38 (m, 4H, C***H***$_2$NH), 2.83 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.49 (m, 4H, COC***H***$_2$), 1.98 (m, 4H, Im-4-CH_2C***H***$_2$), 1.69 (m, 4H, (C***H***$_2$)$_2$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.42 (m, 2H, C***H***$_2$); $C_{69}H_{78}N_{10}O_6$ (1143.42).

N^1,N^8-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}octanediamide (4.16a)

The title compound was prepared from octanedioic acid (70 mg, 0.4 mmol), EDAC (150 mg, 0.8 mmol), HOBt-monohydrate (110 mg, 0.8 mmol), DIEA (0.14 ml, 0.8 mmol) in 5 ml DCM/abs and **4.8** (420 mg, 0.8 mmol) in 5 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **4.16a** (170 mg, 36 %) as yellow-brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35-7.17 (m, 32H, Im-2-***H***, CPh_3), 6.52 (m, 2H, Im-5-***H***), 3.46 (m, 4H, C***H***$_2$NH), 2.61 (m, 4H, Im-4-C***H***$_2$), 2.35 (m, 4H, COC***H***$_2$), 1.89 (m, 4H, Im-4-CH_2C***H***$_2$), 1.63 (m, 4H, $COCH_2$C***H***$_2$), 1.51 (s, 18H, C(C***H***$_3$)$_3$), 1.32 (m, 4H, (C***H***$_2$)$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1157 (MH^+, 100); $C_{70}H_{80}N_{10}O_6$ (1157.45).

N^1,N^9-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}nonanediamide (4.17a)

The title compound was prepared from nonanedioic acid (95 mg, 0.5 mmol), EDAC (190 mg, 1.0 mmol), HOBt-monohydrate (155 mg, 1.0 mmol), DIEA (0.17 ml, 1.0 mmol) in 5 ml DCM/abs and **4.8** (510 mg, 1.0 mmol) in 5 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **4.17a** (470 mg, 80 %) as yellow-brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.37-7.12 (m, 32H, Im-2-***H***, CPh_3), 6.57 (m, 2H, Im-5-***H***), 3.39 (m, 4H, C***H***$_2$NH), 2.83 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.47 (m, 4H, COC***H***$_2$), 1.98 (m, 4H, Im-4-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.37 (m, 6H, (C***H***$_2$)$_3$); $C_{71}H_{82}N_{10}O_6$ (1171.47).

N^1,N^{16}-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}hexadecanediamide (4.18a)

The title compound was prepared from hexadecanedioic acid (90 mg, 0.33 mmol), EDAC (126 mg, 0.66 mmol), HOBt-monohydrate (100 mg, 0.66 mmol), DIEA (0.11 ml, 0.66 mmol) in 5 ml DCM/abs and **4.8** (340 mg, 0.66 mmol) in 5 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **4.18a** (380 mg, 88 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1270 (MH^+, 100); $C_{78}H_{96}N_{10}O_6$ (1269.66).

N^1,N^{22}-Bis{(*tert*-butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}docosanediamide (4.19a)

The title compound was prepared from docosanedioic acid (93 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.08 ml, 0.5 mmol) in 5 ml DCM/abs and **4.8** (255 mg, 0.5 mmol) in 5 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 98/2 v/v) yielding **4.19a** (150 mg, 44 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1354.2 (MH^+, 20), 677.4 ($(M+2H)^{2+}$, 100); $C_{84}H_{108}N_{10}O_6$ (1353.82).

N^1,N^{10}-Bis{amino[3-(1-trityl-1*H*-1,2,4-triazol-5-yl)propylamino]-methylene}decanediamide (4.24a)

To a solution of CDI (195 mg, 1.2 mmol) in DMF (7 ml), decanedioic acid (100 mg, 0.5 mmol) was added and the mixture was stirred under argon for 1 h. In a second flask, **4.23**[28] (410 mg, 1 mmol) and NaH (60 % dispersion in oil) (80 mg, 2 mmol) in DMF (7 ml) under argon was heated to 30-35 °C for 45 min and was then allowed to cool to room temperature. The two mixtures were combined and stirred for 5 h at ambient temperature. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography ($CHCl_3$/MeOH/NH_3 95/3/2 v/v/v) to obtain **4.24a** (300mg, 60 %) as pale white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 8.01 (s, 2H, Triaz-3-***H***), 7.37-7.05 (m, 30H, CPh_3), 3.14 (t, ^3J = 7.6 Hz, 4H, C***H***$_2$NH), 2.88 (m, 4H, Triaz-5-C***H***$_2$), 2.41 (t, ^3J = 7.5 Hz, 4H, COC***H***$_2$), 1.96 (m, 4H, Triaz-5-CH_2C***H***$_2$), 1.63 (m, 4H, $COCH_2$C***H***$_2$), 1.29 (m, 8H, (C***H***$_2$)$_4$). ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 987.7 (MH^+, 10), 494.4 ($(M+2H)^{2+}$, 100); $C_{60}H_{66}N_{12}O_2$ (987.25).

4.5.1.4 Preparation of the deprotected acylguanidines 4.9-4.19 and 4.24

General procedure

To a solution of the protected acylguanidines **4.9a-4.19a** and **4.24a** in DCM/abs was added TFA (20 %) and stirred at ambient temperature until the protecting groups (Boc, Trt) were removed (3-5 h) (TLC control). Subsequently, the solvent was removed *in vacuo* and the residue was purified by preparative RP-HPLC. All compounds were obtained as trifluoroacetic acid salts.

N^1,N^9-Bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}nonanediamide (4.9)

The title compound was prepared from **4.9a** (180 mg, 0.18 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **4.9** (100 mg, 54 %) as colorless

foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^{3}J = 7.1 Hz, 4H, C***H***$_2$NH), 2.71 (t, ^{3}J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.47 (t, ^{3}J = 7.7 Hz, 4H, COC***H***$_2$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.37 (m, 6H, (C***H***$_2$)$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.38 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 157.13 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.44 (quat. Thiaz-5-***C***), 41.60 (-, ***C***H$_2$NH), 37.74 (-, CO***C***H$_2$), 29.82 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.40 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 11.45 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{25}H_{42}N_{10}O_2S_2$ + H]$^{+}$) calcd. 579.3012, found 579.3010; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50); anal. HPLC: k`= 2.08 (t_R = 10.22 min, column A), purity = 92 %; $C_{25}H_{42}N_{10}O_2S_2$ · 4TFA (1034.37).

***N*^{1},*N*^{8}-Bis{[3-(2-aminothiazol-5-yl)propylamino](amino)methylene}octanediamide (4.10)**

The title compound was prepared from **4.10a** (170 mg, 0.18 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **4.10** (160 mg, 90 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^{3}J = 7.1 Hz, 4H, C***H***$_2$NH), 2.77 (t, ^{3}J = 7.1 Hz, 4H, Thiaz-5-C***H***$_2$), 2.48 (t, ^{3}J = 7.4 Hz, 4H, COC***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.67 (m, 4H, COCH$_2$C***H***$_2$), 1.39 (m, 4H, (C***H***$_2$)$_2$). ^{13}C-NMR (CD_3OD) δ (ppm): 176.47 (quat. ***C***=O), 172.43 (quat. Thiaz-2-***C***), 155.92 (quat. ***C***=NH), 125.54 (quat. Thiaz-5-***C***), 123.27 (+, Thiaz-4-***C***), 40.66 (-, ***C***H$_2$NH), 36.83 (-, CO***C***H$_2$), 28.80 (-, ***C***H$_2$), 28.74 (-, Thiaz-5-CH$_2$-***C***H$_2$), 24.40 (-, Thiaz-5-***C***H$_2$), 24.08 (-, COCH$_2$***C***H$_2$). HRLSIMS: *m/z* for ([$C_{22}H_{36}N_{10}O_2S_2$ + H]$^{+}$) calcd. 537.2542, found 537.2546; prep. HPLC: MeCN/0.1% TFA/aq (10/90-50/50); anal. HPLC: k`= 1.61 (t_R = 8.66 min, column A), purity = 95 %; $C_{22}H_{36}N_{10}O_2S_2$ · 4TFA (992.33).

***N*^{1},*N*^{10}-Bis{[3-(2-aminothiazol-5-yl)propylamino](amino)methylene}decanediamide (4.11)**

The title compound was prepared from **4.11a** (200 mg, 0.2 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **4.11** (100 mg, 49 %) as yellow-brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^{3}J = 7.1 Hz, 4H, C***H***$_2$NH), 2.77 (t, ^{3}J = 7.1 Hz, 4H, Thiaz-5-C***H***$_2$), 2.47 (t, ^{3}J = 7.4 Hz, 4H, COC***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$). ^{13}C-NMR (CD_3OD) δ (ppm): 176.47 (quat. ***C***=O), 172.43 (quat. Thiaz-2-***C***), 155.92 (quat. ***C***=NH), 125.55 (quat. Thiaz-5-***C***), 123.27 (+, Thiaz-4-***C***), 40.66 (-, ***C***H$_2$NH), 36.83 (-, CO***C***H$_2$),

30.43 (-, ***C***H_2), 28.80 (-, ***C***H_2), 28.74 (-, Thiaz-5-CH_2-***C***H_2), 24.41 (-, Thiaz-5-***C***H_2), 24.08 (-, $COCH_2$***C***H_2). HRLSIMS: *m/z* for ($[C_{24}H_{40}N_{10}O_2S_2 + H]^+$) calcd. 565.2855, found 565.2855; prep. HPLC: MeCN/0.1% TFA/aq (10/90-50/50); anal. HPLC: k`= 2.13 (t_R = 10.37 min, column A), purity = 100 %; $C_{24}H_{40}N_{10}O_2S_2 \cdot 4TFA$ (1020.36).

***N*^{1},*N*^{16}-Bis{[3-(2-aminothiazol-5-yl)propylamino](amino)methylene}hexadecane-diamide (4.12)

The title compound was prepared from **4.12a** (150 mg, 0.14 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **4.12** (80 mg, 52 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.1 Hz, 4H, C***H***$_2$NH), 2.77 (t, ^3J = 7.41 Hz, 4H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.41 Hz, 4H, COC***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.37 (m, 8H, (C***H***$_2$)$_4$), 1.29 (m, 12H, (C***H***$_2$)$_6$). ^{13}C-NMR (CD_3OD) δ (ppm): 177.40 (quat. ***C***=O), 171.81 (quat. Thiaz-2-***C***), 155.34 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.37 (+, Thiaz-4-***C***), 41.49 (-, ***C***H_2NH), 37.80 (-, CO***C***H_2), 30.82 (-, ***C***H_2), 30.77 (-, ***C***H_2), 30.64 (-, ***C***H_2), 30.43 (-, ***C***H_2), 30.06 (-, ***C***H_2), 29.51 (-, Thiaz-5-CH_2-***C***H_2), 25.49 (-, Thiaz-5-***C***H_2), 24.89 (-, $COCH_2$***C***H_2). HRLSIMS: *m/z* for ($[C_{30}H_{52}N_{10}O_2S_2 + H]^+$) calcd. 649.3794, found 649.3779; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50); anal. HPLC: k`= 3.64(t_R = 15.40 min, column A), purity = 99 %; $C_{30}H_{52}N_{10}O_2S_2 \cdot 4TFA$ (1104.45).

***N*^{1},*N*^{22}-Bis{[3-(2-aminothiazol-5-yl)propylamino](amino)methylene}docosane-diamide (4.13)

The title compound was prepared from **4.13a** (230 mg, 0.19 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **4.13** (120 mg, 53 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.1 Hz, 4H, C***H***$_2$NH), 2.77 (t, ^3J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 1.96 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.37-1.26 (m, 32H, (C***H***$_2$)$_{16}$). ^{13}C-NMR (CD_3OD) δ (ppm): 177.42 (quat. ***C***=O), 171.83 (quat. Thiaz-2-***C***), 155.35 (quat. ***C***=NH), 126.35 (quat. Thiaz-5-***C***), 123.34 (+, Thiaz-4-***C***), 41.47 (-, ***C***H_2NH), 37.79 (-, CO***C***H_2), 30.84 (-, ***C***H_2), 30.76 (-, ***C***H_2), 30.62 (-, ***C***H_2), 30.42 (-, ***C***H_2), 30.05 (-, ***C***H_2), 29.50 (-, Thiaz-5-CH_2-***C***H_2), 25.49 (-, Thiaz-5-***C***H_2), 24.89 (-, $COCH_2$***C***H_2). HRLSIMS: *m/z* for ($[C_{36}H_{64}N_{10}O_2S_2 + H]^+$) calcd. 733.4733, found 733.4728; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50); anal. HPLC: k`= 5.22 (t_R = 20.63 min, column A), purity = 99 %; $C_{36}H_{64}N_{10}O_2S_2 \cdot 4TFA$ (1188.55).

N^1,N^6-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}hexanediamide (4.14)

The title compound was prepared from **4.14a** (120 mg, 0.10 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.14** (20 mg, 22 %) as pale brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (d, ^4J = 1.1 Hz, 1H, Im-2-***H***), 7.36 (s, 2H, Im-5-***H***), 3.38 (t, ^3J = 6.9 Hz, 4H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.53 (m, 4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.72 (m, 4H, $COCH_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.35 (quat. ***C***=O), 155.41 (quat. ***C***=NH), 134.94 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.15 (+, Im-5-***C***), 41.54 (-, ***C***H_2NH), 37.24 (-, CO***C***H_2), 27.99 (-, Im-4-CH_2***C***H_2), 24.53 (-, $COCH_2$***C***H_2), 22.58 (-, Im-4-***C***H_2); HRLSIMS: *m/z* for ([$C_{20}H_{32}N_{10}O_2$ + H]$^+$) calcd. 445.2788, found 445.2794; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 0.92 (t_R = 6.36 min, column B), purity = 96 %; $C_{20}H_{32}N_{10}O_2$ · 4TFA (900.61).

N^1,N^7-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}heptanediamide (4.15)

The title compound was prepared from **4.15a** (300 mg, 0.26 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.15** (178 mg, 75 %) as colorless foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (s, 1H, Im-2-***H***), 7.36 (s, 2H, Im-5-***H***), 3.38 (t, ^3J = 7.1 Hz, 4H, C***H***$_2$NH), 2.83 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.49 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.69 (m, 4H, $COCH_2$C***H***$_2$), 1.42 (m, 2H, C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.24 (quat. ***C***=O), 155.40 (quat. ***C***=NH), 134.97 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 41.54 (-, ***C***H_2NH), 37.46 (-, CO***C***H_2), 29.20 (-, $COCH_2CH_2$***C***H_2), 27.96 (-, Im-4-CH_2***C***H_2), 25.02 (-, $COCH_2$***C***H_2), 22.55 (-, Im-4-***C***H_2); HRLSIMS: *m/z* for ([$C_{21}H_{34}N_{10}O_2$ + H]$^+$) calcd. 459.2944, found 459.2955; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 1.11 (t_R = 7.01 min, column A), purity = 90 %; $C_{21}H_{34}N_{10}O_2$ · 4TFA (914.64).

N^1,N^8-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}octanediamide (4.16)

The title compound was prepared from **4.16a** (150 mg, 0.13 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.16** (28 mg, 23 %) as pale brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (s, 2H, Im-2-***H***), 7.36 (s, 2H, Im-5-***H***), 3.38 (t, ^3J = 6.9 Hz, 4H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.48 (t, ^3J = 7.4 Hz,

4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.67 (m, 4H, $COCH_2C$***H***$_2$), 1.36 (m, 4H, (C***H***$_2)_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.35 (quat. ***C***=O), 155.41 (quat. ***C***=NH), 134.96 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 41.54 (-, ***C***H_2NH), 37.64 (-, CO***C***H_2), 29.60 (-, $COCH_2CH_2$***C***H_2), 27.97 (-, Im-4-CH_2***C***H_2), 25.22 (-, $COCH_2$***C***H_2), 22.55 (-, Im-4-***C***H_2); HRLSIMS: *m/z* for ($[C_{22}H_{36}N_{10}O_2 + H]^+$) calcd. 473.3101, found 473.3108; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 1.35 (t_R = 7.81 min, column A), purity = 95 %; $C_{22}H_{36}N_{10}O_2 \cdot 4$TFA (928.67).

***N*^{1},*N*^{9}-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}nonanediamide (4.17)

The title compound was prepared from **4.17a** (150 mg, 0.13 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.17** (10 mg, 10 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.81 (s, 2H, Im-2-***H***), 7.37 (s, 2H, Im-5-***H***), 3.39 (t, ^3J = 7.2 Hz, 4H, C***H***$_2$NH), 2.83 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.47 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2C$***H***$_2$), 1.37 (m, 4H, (C***H***$_2)_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.14 (quat. ***C***=O), 155.40 (quat. ***C***=NH), 134.97 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 41.55 (-, ***C***H_2NH), 37.71 (-, CO***C***H_2), 29.81 (-, $COCH_2CH_2$***C***H_2), 27.77 (-, Im-4-CH_2***C***H_2), 25.39 (-, $COCH_2$***C***H_2), 22.56 (-, Im-4-***C***H_2); HRLSIMS: *m/z* for ($[C_{23}H_{38}N_{10}O_2 + H]^+$) calcd. 487.3257, found 487.3246; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 1.63 (t_R = 8.74 min, column A), purity = 90 %; $C_{23}H_{38}N_{10}O_2 \cdot 4$TFA (942.69).

***N*^{1},*N*^{16}-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}hexadecane-diamide (4.18)

The title compound is was prepared from **4.18a** (200 mg, 0.16 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.18** (50 mg, 30 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.81 (d, ^4J = 1.37 Hz, 2H, Im-2-***H***), 7.37 (s, 2H, Im-5-***H***), 3.38 (t, ^3J = 6.861 Hz, 4H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 4H, Im-4-C***H***$_2$), 2.47 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2C$***H***$_2$), 1.38–1.27 (m, 20H, (C***H***$_2)_{10}$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.43 (quat. ***C***=O), 134.98 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.12 (+, Im-5-***C***), 41.56 (-, ***C***H_2NH), 37.79 (-, CO***C***H_2), 30.82 (-, ***C***H_2), 30.77 (-, ***C***H_2), 30.63 (-, ***C***H_2), 30.43 (-, $COCH_2CH_2CH_2$***C***H_2), 30.06 (-, $COCH_2CH_2$***C***H_2), 27.96 (-, Im-4-CH_2***C***H_2), 25.50 (-, $COCH_2$***C***H_2), 22.55 (-, Im-4-***C***H_2); HRLSIMS: *m/z* for ($[C_{30}H_{52}N_{10}O_2 + H]^+$) calcd. 585.4353, found 585.4350; prep.

HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 3.44 (t_R = 14.75 min, column A), purity = 99 %; $C_{30}H_{52}N_{10}O_2 \cdot 4TFA$ (1040.88).

***N*^{1},*N*^{22}-Bis{[3-(1*H*-imidazol-4-yl)propylamino](amino)methylene}docosanediamide (4.19)**

The title compound is was prepared from **4.19a** (150 mg, 0.12 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.19** (25 mg, 31 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.82 (d, ^{4}J = 1.10 Hz, 2H, Im-2-***H***), 7.37 (s, 2H, Im-5-***H***), 3.39 (t, ^{3}J = 6.861 Hz, 4H, C***H***$_2$NH), 2.84 (t, ^{3}J = 7.41 Hz, 4H, Im-4-C***H***$_2$), 2.47 (t, ^{3}J = 7.41 Hz, 4H, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.34–1.26 (m, 32H, (C***H***$_2$)$_{16}$); ^{13}C-NMR (CD_3OD, 400 MHz, HSQC, HMQC) δ (ppm): 177.32 (quat. ***C***=O), 135.00 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.08 (+, Im-5-***C***), 41.60 (-, ***C***H$_2$NH), 37.79 (-, CO***C***H$_2$), 30.85 (-, ***C***H$_2$), 30.81 (-, ***C***H$_2$), 30.76 (-, ***C***H$_2$), 30.61 (-, ***C***H$_2$), 30.42 (-, COCH$_2$CH$_2$CH$_2$***C***H$_2$), 30.05 (-, COCH$_2$CH$_2$***C***H$_2$), 27.93 (-, Im-4-CH$_2$***C***H$_2$), 25.45 (-, COCH$_2$***C***H$_2$), 22.55 (-, Im-4-***C***H$_2$); HRLSIMS: *m/z* for ([$C_{36}H_{64}N_{10}O_2$ + H]$^{+}$) calcd. 669.5292, found 669.5291; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 4.91 (t_R = 19.61 min, column A), purity = 96 %; $C_{36}H_{64}N_{10}O_2 \cdot 4TFA$ (1125.04).

***N*^{1},*N*^{10}-Bis{[3-(1*H*-1,2,4-triazol-5-yl)propylamino](amino)methylene}decanediamide (4.24)**

The title compound was prepared from **4.24a** (300 mg, 0.31 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **4.24** (85 mg, 29 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.54 (s, 2H, Triaz-3-***H***), 3.42 (t, ^{3}J = 7.2 Hz, 4H, C***H***$_2$NH), 2.94 (t, ^{3}J = 7.4 Hz, 4H, Triaz-5-C***H***$_2$), 2.46 (t, ^{3}J = 7.4 Hz, 4H, COC***H***$_2$), 2.11 (m, 4H, Triaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.36 (m, 4H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.22 (quat. ***C***=O), 163.55 (quat. Triaz-5-***C***), 155.41 (quat. ***C***=NH), 138.37 (+, Triaz-3-***C***), 41.62 (-, ***C***H$_2$NH), 37.79 (-, CO***C***H$_2$), 30.19 (-, ***C***H$_2$), 29.96 (-, ***C***H$_2$), 26.90 (-, Triaz-5-CH$_2$***C***H$_2$), 25.41 (-, COCH$_2$***C***H$_2$), 24.11 (-, Triaz-5-***C***H$_2$). HRLSIMS: *m/z* for ([$C_{22}H_{38}N_{12}O_2$ + H]$^{+}$) calcd. 503.3319, found 503.3304; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50); anal. HPLC: k`= 1.77 (t_R = 7.41 min, column B), purity = 100 %; $C_{22}H_{38}N_{12}O_2 \cdot 4TFA$ (958.7).

4.5.1.5 Separation of the deprotected acylguanidines 4.20-4.22

6-{[3-(1*H*-imidazol-4-yl)propylamino](amino)methyleneamino}-6-oxohexanoic acid (4.20)

The title compound was separated as side-product during the purification of **4.14** by preparative HPLC. **4.20** (5 mg, 10 µmol) was obtained as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 8.78 (d, ^4J = 1.1 Hz, 1H, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.39 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 2H, Im-4-C***H***$_2$), 2.51 (t, ^3J = 6.9, 2H, COC***H***$_2$), 2.33 (t, ^3J = 6.9, 2H, C***H***$_2$COOH), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.67 (m, 4H, COCH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.02 (quat. ***C***=O), 155.34 (quat. ***C***=NH), 135.04 (+, Im-2-***C***), 134.44 (quat. Im-4-***C***), 117.11 (+, Im-5-***C***), 41.62 (-, ***C***H$_2$NH), 37.37 (-, CO***C***H$_2$), 34.47 (-, ***C***H$_2$COOH), 27.98 (-, Im-4-CH$_2$***C***H$_2$), 25.25 (-, COCH$_2$***C***H$_2$), 24.83 (-, ***C***H$_2$CH$_2$COOH), 22.63 (-, Im-4-***C***H$_2$); HRLSIMS: *m/z* for ([$C_{13}H_{21}N_5O_3$ + H]$^+$) calcd. 296.1723, found 296.1731; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 0.53 (t_R = 4.09 min, column B), purity = 100 %; $C_{13}H_{21}N_5O_3 \cdot 2$TFA (523.38).

8-{[3-(1*H*-imidazol-4-yl)propylamino](amino)methyleneamino}-8-oxooctanoic acid(4.21)

The title compound was separated as side-product during the purification of **4.16** by preparative HPLC. **4.21** (12 mg, 22 µmol) was obtained as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 8.82 (d, ^4J = 1.4 Hz, 1H, Im-2-***H***), 7.37 (s, 1H, Im-5-***H***), 3.39 (t, ^3J = 7.1 Hz, 2H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 2H, Im-4-C***H***$_2$), 2.48 (t, ^3J = 7.4, 2H, COC***H***$_2$), 2.29 (t, ^3J = 7.1, 2H, C***H***$_2$COOH), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$), 1.38 (m, 4H, (C***H***$_2$)$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.62 (quat. ***C***=O), 177.32 (quat. ***C***=O), 149.12 (quat. ***C***=NH), 135.01 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 41.58 (-, ***C***H$_2$NH), 37.67 (-, CO***C***H$_2$), 34.81 (-, ***C***H$_2$COOH), 29.83 (-, ***C***H$_2$), 29.60 (-, ***C***H$_2$), 27.97 (-, Im-4-CH$_2$***C***H$_2$), 25.84 (-, COCH$_2$***C***H$_2$), 25.22 (-, ***C***H$_2$CH$_2$COOH), 22.55 (-, Im-4-***C***H$_2$); HRLSIMS: *m/z* for ([$C_{15}H_{25}N_5O_3$ + H]$^+$) calcd. 324.2036, found 324.2037; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 1.42 (t_R = 6.48 min, column B), purity = 91 %; $C_{15}H_{25}N_5O_3 \cdot 2$TFA (551.43).

22-{[3-(1*H*-imidazol-4-yl)propylamino](amino)methyleneamino}-22-oxodocosanoic acid (4.22)

The title compound was separated as side-product during the purification of **4.19** by preparative HPLC. **4.22** (10 mg, 13 µmol) was obtained as white foam-like solid. ^{1}H-

NMR (CD_3OD) δ (ppm): 8.80 (d, ^4J = 1.1 Hz, 1H, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.39 (t, ^3J = 7.1 Hz, 2H, C***H***$_2$NH), 2.83 (t, ^3J = 7.7 Hz, 2H, Im-4-C***H***$_2$), 2.47 (t, ^3J = 7.4, 2H, COC***H***$_2$), 2.27 (t, ^3J = 7.1, 2H, C***H***$_2$COOH), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.62 (m, 4H, COCH$_2$C***H***$_2$), 1.29 (m, 32H, (C***H***$_2$)$_{16}$); HRLSIMS: *m/z* for ($[C_{29}H_{53}N_5O_3 + H]^+$) calcd. 520.4223, found 520.4218; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 4.39 (t_R = 15.57 min, column B), purity = 97 %; $C_{29}H_{53}N_5O_3 \cdot 2TFA$ (747.8).

4.5.2 Pharmacological methods

4.5.2.1 Materials

See section 3.5.2.1

4.5.2.2 Determination of histamine receptor agonism and antagonism in GTPase and GTPγS binding assays

Generation of recombinant baculoviruses, cell culture and membrane preparation

Recombinant baculoviruses encoding human H_1R, a fusion protein of the human H_2R with $Gs\alpha_S$, a fusion protein of the guinea pig H_2R with $Gs\alpha_S$, the human H_3R, a fusion protein of the human H_4R with RGS19 as well as four fusion proteins of mutant H_2Rs with $Gs\alpha_S$ (hH_2R-C17Y-A271D-$Gs\alpha_S$, hH_2R-C17Y-$Gs\alpha_S$, hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$) were prepared as described,[30,32-33,58] using the BaculoGOLD transfection kit (BDPharmingen, San Diego, CA) according to the manufacturer's instructions.

Sf9 cells were cultured in 250 or 500 ml disposable Erlenmeyer flasks at 28 °C under rotation at 150 r.p.m in Insect-Xpress medium (Lonza, Velviers, Belgium) supplemented with 5 % (v/v) fetal calf serum (Biochrom, Berlin, Germany) and 0.1 mg/ml gentamicin (Lonza, Walkersville, MD). Cells were maintained at a density of 0.5 – 6.0 x 10^6 cells/ml. After initial transfection, high-titer virus stocks were generated by two sequential virus amplifications. In the first amplification, cells were seeded at 2.0 x 10^6 cells/ml and infected with a 1:100 dilution of the supernatant from the initial transfection. Cells were cultured for 7 days, resulting in the lysis of the entire cell population. The supernatant fluid of this infection was harvested and stored under light protection at 4 °C. In a second amplification, cells were seeded at 3.0 x 10^6 cells/ml and infected with a 1:20 dilution of the supernatant fluid from the first amplification. Cells were cultured for 48 h, and the

supernatant fluid was harvested. After a 48 h culture period, the majority of cells showed signs of infections (e.g. altered morphology, viral inclusion bodies), whereas most of the cells were still intact. The supernatant from the second amplification was stored under light protection at 4 °C and used as routine virus stock for membrane preparations. For membrane preparation, cells were sedimented by centrifugation (1000 rpm, 5 min, rt) and suspended in fresh medium at 3.0 x 10^6 cells/ml. Cells were infected with 1:100 dilutions of high-titer baculovirus stocks encoding the various histamine receptors, histamine receptor fusion proteins, G-protein subunits and RGS proteins. Cells were cultured for 48 h before membrane preparation. Sf9 membranes were prepared as described,[59] using 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 10 µg/ml benzamidine and 10 µg/ml leupeptin as protease inhibitors. Membranes were suspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4) and stored at -80 °C until use. Protein concentrations were determined using the DC protein assay kit (Bio-Rad, München, Germany).

Steady-state GTPase activity assay with Sf9 insect cell membranes expressing histamine H_1-H_4 receptors and H_2R mutants

Membranes were thawed, sedimented and resuspended in 10 mM Tris/HCl, pH 7.4. In the case of the H_1R and H_2R, Sf9 membranes expressing either H_1R isoforms plus RGS4 or H_2R-Gsα_S fusion proteins, respectively, were used.[30,38] H_3R-regulated GTP hydrolysis was determined with membranes co-expressing human H_3R, mammalian Gα_{i2}, G$\beta_1\gamma_2$ and RGS4. Human H_4R activity was measured with membranes co-expressing an H_4R-RGS19 fusion protein with Gα_{i2} and G$\beta_1\gamma_2$. Activity on H_2R mutants was measured with hH_2R-C17Y-A271D-Gsα_S, hH_2R-C17Y-Gsα_S, hH_2R-gpE2-Gsα_S and gpH_2R-hE2-Gsα_S fusion proteins, respectively.[32-33] Assay tubes contained Sf9 membranes (10-20 µg of protein/tube), $MgCl_2$ (H_1R, H_2R: 1.0 mM; H_3R, H_4R: 5.0 mM), 100 µM EDTA, 100 µM ATP, 100 nM GTP, 100 µM adenylyl imidodiphosphate, 5 mM creatine phosphate, 40 µg creatine kinase and 0.2 % (w/v) bovine serum albumin in 50 mM Tris/HCl, pH 7.4, as well as ligands at various concentrations. In H_4R assays, NaCl (final concentration of 100 mM) was included. Reaction mixtures (80 µl) were incubated for 2 min at 25 °C before the addition of 20 µl [γ-^{32}P]GTP (0.1 µCi/tube) or [γ-^{33}P]GTP (0.05 µCi/tube). Reactions were run for 20 min at 25 °C and terminated by the addition of 900 µl of slurry consisting of 5% (w/v) activated charcoal suspended in 50 mM NaH_2PO_4, pH 2.0. Charcoal absorbs nucleotides but not P_i. Charcoal-quenched reaction mixtures were centrifuged for 7 min at

room temperature at 13.000 *g*. 600 µl of the supernatant fluid were removed and $^{32}P_i$ or $^{33}P_i$ was determined by Cerenkov or liquid scintillation counting, respectively. Enzyme activities were corrected for spontaneous degradation of [γ-^{32}P]GTP or [γ-^{33}P]GTP, respectively, determined in tubes containing all components described above, plus a high concentration of unlabeled GTP (1 mM) to prevent enzymatic hydrolysis of the labelled nucleotides in the presence of Sf9 membranes. Spontaneous [γ-^{32}P]GTP or [γ-^{33}P]GTP hydrolysis was <1 % of the total amount of radioactivity added. The experimental conditions chosen ensured that not more than 10 % of the total amount of added [γ-^{32}P]GTP and [γ-^{33}P]GTP was converted to $^{32}P_i$ and $^{33}P_i$, respectively. All experimental data were analyzed by non-linear regression with the Prism 5 program (GraphPad Software, San Diego, CA).

[^{35}S]GTPγS Binding Assay

[^{35}S]GTPγS Binding Assays[60-61] were performed as previously described for the H_2R[58,62] using Sf9 insect cell membranes expressing the gpH_2R-Gsα_S fusion protein. The respective membranes were thawed and sedimented by a 10 min centrifugation at 4 °C and 13,000*g*. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each assay tube contained Sf9 membranes (15 - 30 µg protein/tube), 1 µM GDP, 0.05% (w/v) bovine serum albumin, 0.2 nM [^{35}S]GTPγS and the investigated ligands at various concentrations in binding buffer (total volume 250 µl). Incubations were conducted for 90 min at 25 °C and shaking at 250 rpm. Bound [^{35}S]GTPγS was separated from free [^{35}S]GTPγS by filtration through GF/C filters, followed by three washes with 2 ml of binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting. The experimental conditions chosen ensured that no more than 10% of the total amount of [^{35}S]GTPγS added was bound to filters. Non-specific binding was determined in the presence of 10 µM unlabeled GTPγS.

4.5.2.3 Histamine H_2 receptor assay on isolated guinea pig right atrium

See section 3.5.2.3

4.6 References

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Chapter 5

Heterobivalent motifs and variations of the spacer in histamine H_2 receptor agonists

5.1 Introduction

The term “bivalent ligand” describes molecules containing two sets of pharmacophoric entities linked through a spacer. Thereby, the two pharmacophoric moieties can be identical to form homobivalent compounds (twin compounds) or in case of heterobivalent compounds consist of two different recognition units.[1-3] In the broader sense bivalent ligands can be divided in molecules containing two sets of pharmacophoric groups or a single pharmacophore connected to a non-pharmacophoric recognition unit.[4-5] Over the past few decades, bivalent ligands have been developed for a variety of G-protein coupled receptors (GPCRs), including opioid,[5-6] serotonin,[7-9] dopamine,[9] adrenergic[10] and muscarinergic receptors.[11-13] Previously considered as monomeric polypeptides, GPCRs have been shown to exist and function as dimers or oligomers,[14-15] yet many of the most potent bivalent ligands have relatively short spacers, suggesting that the compounds interact with neighboring binding sites on a single receptor protomer.[8,16-17]

Among the different tools offered to medicinal chemists to design potent and selective GPCR agonists and antagonists, the bivalent ligand approach has proven to be valuable to improve potency, selectivity and efficacy as well as the pharmacokinetic profile of compounds.[18] Likewise, the application of the bivalent ligand approach to acylguanidine-type histamine H_2R agonists described in chapter 4 resulted in highly potent and selective histamine H_2R agonists. After the successful preparation of symmetrical bivalent hetarylpropylguanidines with alkyl spacers of various lengths (6-27 Å), the present study was focused on the chemical nature of the spacer as well as on unsymmetrical bivalent

ligands bearing two different sets of pharmacophoric groups. Besides promising applications to improve the pharmacological profile of H_2R agonists, bivalent ligands were synthesized as pharmacological tools with the hope of expanding our knowledge of the structure-activity relationships (SAR) of bivalent acylguanidine-type ligands and of the topology of the putative accessory binding site at histamine H_2 receptors.

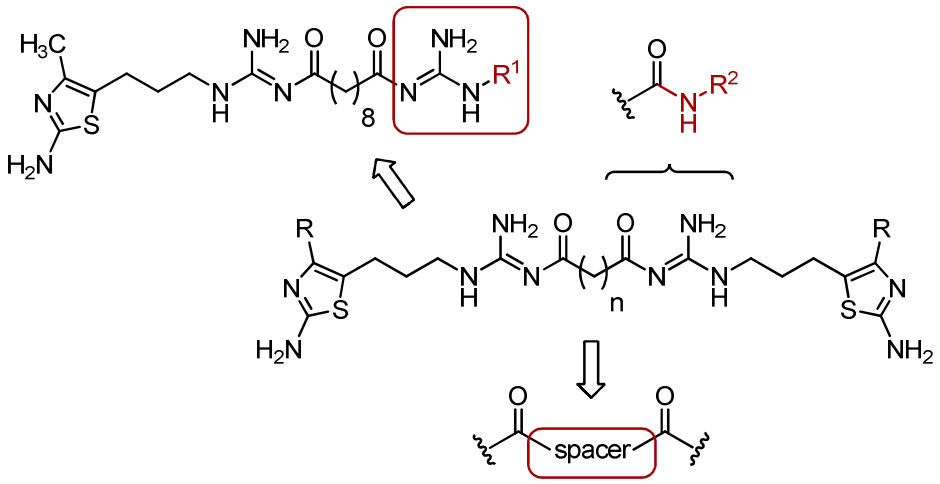


Figure 5.1. Overview of the structural modifications of bivalent acylguanidine-type H_2R agonists. R: H, CH_3; R^1: alkyl, arylalkyl, hetarylpropyl, aminoalkyl; R^2: 2-amino-4-methylthiazolylpropyl, (piperidino)-methylphenoxypropylamine; spacer: alkyl chains containing disulfide, ether, amide or phenylene groups, branched linkers.

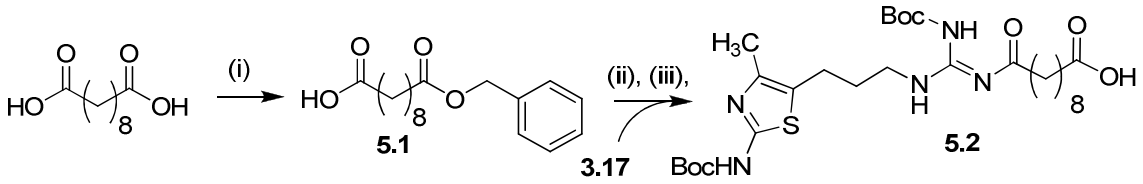
5.2 Chemistry

The title compounds were preferentially synthesized according to the synthetic routes described in chapters 3 and 4. The synthetic strategies aimed at compounds of maximal purity on the low mg scale rather than at the optimization of yields and synthetic pathways.

Synthesis of unsymmetrical bivalent ligands

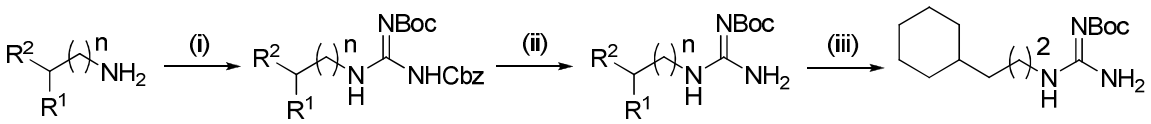
The synthesis of unsymmetrical bivalent acylguanidines with two different pharmacophoric moieties (**5.26**-**5.42**) started with the preparation of 10-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}aminomethylene)-10-oxodecanoic acid (**5.2**). To reduce the formation of by-products, one carboxylic function of the dicarboxylic acid was capped with a benzyl group, and the resulting 10-benzyloxy-10-oxodecanoic acid (**5.1**) was coupled to the Boc-protected 3-(2-

amino-4-methylthiazol-5-yl)propylguanidine building block **3.17**. The hydrogenolysis of the benzyl ester group resulted in the key intermediate **5.2**.



Scheme 5.1. Synthesis of 10-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}aminomethylene)-10-oxodecanoic acid (**5.2**). Reagents and conditions: (i) BnOH (1 eq), DCC (1.2 eq), DMAP (cat.), THF/abs, 48 h, rt; (ii) EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 16 h, rt; (iii) H_2, Pd/C (10 %), MeOH, 1 h, rt.

Guanidinylation of commercially available amines with the isothiourea derivative **3.3** in the presence of $HgCl_2$, followed by the deprotection of the Cbz group by a reduction step, classically carried out by hydrogenation over Pd/C catalyst, afforded the mono Boc-protected guanidine building blocks **5.14-5.24**. In addition, the Boc-protected (3-cyclohexylpropyl)guanidine **5.25** was prepared from the corresponding (3-phenylpropyl)guanidine **5.14** by hydrogenation of the phenyl ring over Rh/Al_2O_3 catalyst in MeOH.[19]

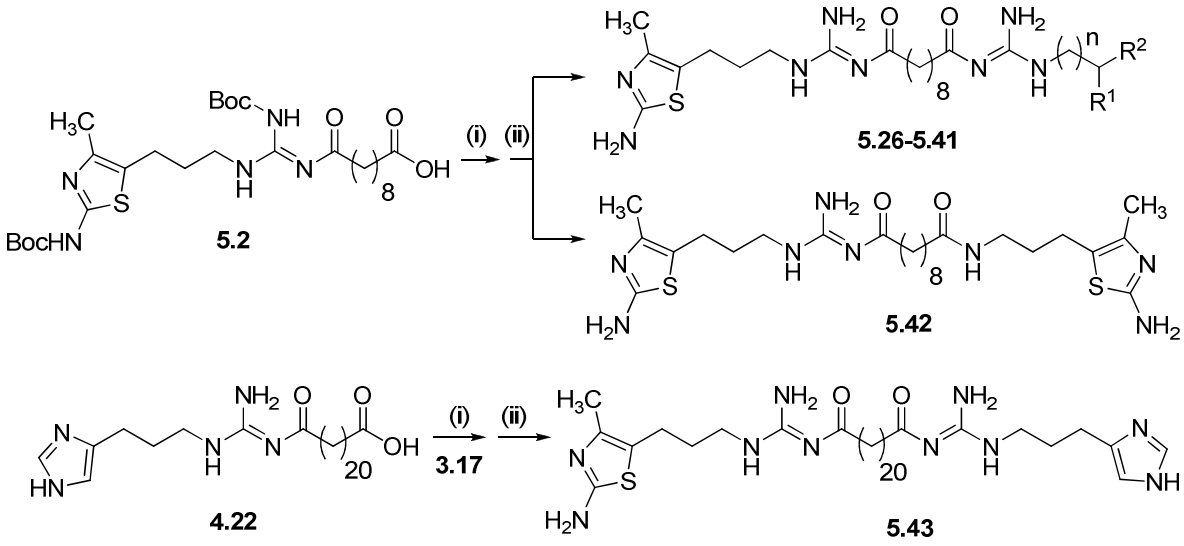


Compd.	R^1	R^2	n	Compd.	R^1	R^2	n	Compd.	R^1	R^2	n
5.3, 5.14	H	Ph	2	**5.7, 5.18**	(*R*)-CH_3	Ph	0	**5.11, 5.22**	H	CH_3	1
5.4, 5.15	Ph	Ph	2	**5.8, 5.19**	H	4-OMe-Ph	0	**5.12, 5.23**	CH_3	CH_3	1
5.5, 5.16	H	Ph	0	**5.9, 5.20**	H	3,4-OMe-Ph	0	**5.13, 5.24**	H	NHBoc	2
5.6, 5.17	(*S*)-CH_3	Ph	0	**5.10, 5.21**	H	H	0				

Scheme 5.2. General procedure for the preparation of Boc-protected guanidines **5.14**-**5.25**. Reagents and conditions: (i) **3.3** (1 eq), $HgCl_2$ (2 eq), NEt_3 (3 eq), DCM/abs, 48 h, rt; (ii) H_2, Pd/C (10 %), MeOH/THF (1:1), 8 bar, 3-5 d, rt; (iii) **5.14** (1 eq), H_2, Rh/Al_2O_3 (cat.), MeOH, 7 bar, 4 d, rt.

To obtain the designated unsymmetrical bivalent ligands **5.26-5.42**, the Boc-protected guanidines **3.18**, **4.8** and **5.14-5.25**, the unprotected guanidines **4.23**[20] and 3-phenylbutan-1-ylguanidine,[21] and the *tert*-butyl 5-(3-aminopropyl)-4-methylthiazol-2-ylcarbamate **3.13**, respectively, were coupled to **5.2** by *N*-acylation using EDAC, HOBt and DIEA as standard coupling reagents to yield the protected compounds **5.26a-5.42a**. Finally,

removal of the protecting groups under acidic conditions gave the unsymmetrical acylguanidines **5.26-5.42** (Scheme 5.3), which were purified by preparative RP-HPLC. In addition, the synthesis of the unsymmetrical compound **5.43**, containing a docosanedioyl spacer was achieved by coupling **4.22** with **3.17** under similar conditions.



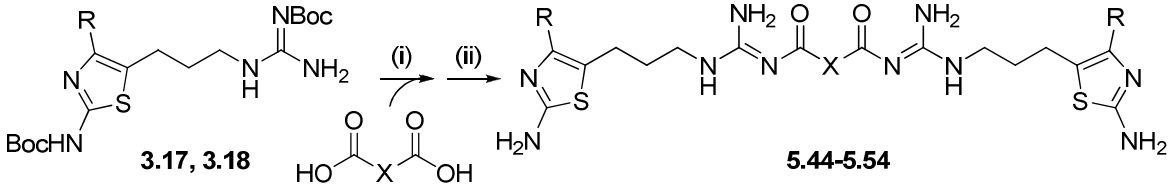
Compd.	R^1	R^2	n	Compd.	R^1	R^2	n
5.26	H	2-aminothiazol-5-yl	2	**5.34**	(*S*)-CH_3	Ph	0
5.27	H	imidazol-4-yl	2	**5.35**	(*R*)-CH_3	Ph	0
5.28	H	1,2,4-triazol-5-yl	2	**5.36**	H	4-OMe-Ph	0
5.29	H	Ph	2	**5.37**	H	3,4-OMe-Ph	0
5.30	CH_3	Ph	2	**5.38**	H	H	0
5.31	Ph	Ph	2	**5.39**	H	CH_3	1
5.32	H	cHex	2	**5.40**	CH_3	CH_3	1
5.33	H	Ph	0	**5.41**	H	NH_2	2

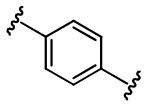
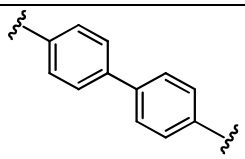
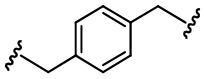
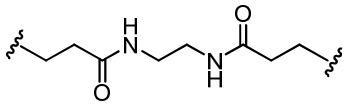
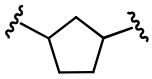
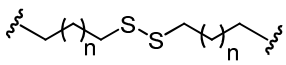
Scheme 5.3. General procedure for the preparation of unsymmetrical bivalent acylguanidines **5.26-5.43**. Reagents and conditions: (i) for **5.26**, **5.27** and **5.29-5.43**: **3.13**, **3.17**, **3.18**, **4.8**, 3-phenylbutan-1-ylguanidine[21] or **5.14-5.25** (1 eq), EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 16 h, rt; for **5.28**: **4.23**[20] (1 eq), CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), DMF, 5 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt.

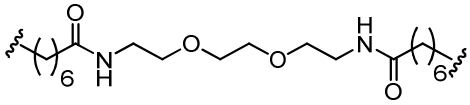
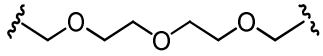
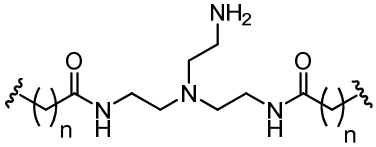
Structural modifications of the spacer

As depicted in Scheme 5.4, various structural moieties including ether, amide, phenylene and disulfide groups as well as *N*,*N*-bis(2-aminoethyl)ethane-1,2-diamine (branched linkers) were incorporated into the spacer. By analogy with the procedures applied to the preparation of homobivalent acylguanidines (see chapter 4), coupling of two equivalents

of Boc-protected aminothiazolylpropylguanidines **3.17** or **3.18** with one equivalent of the pertinent dicarboxylic acid, followed by deprotection, was feasible to synthesize a small library of symmetrical acylguanidine-type H_2R agonists with spacers of various chemical compositions. Whereas most of the spacers were commercially available, long spacers were individually synthesized.[22]

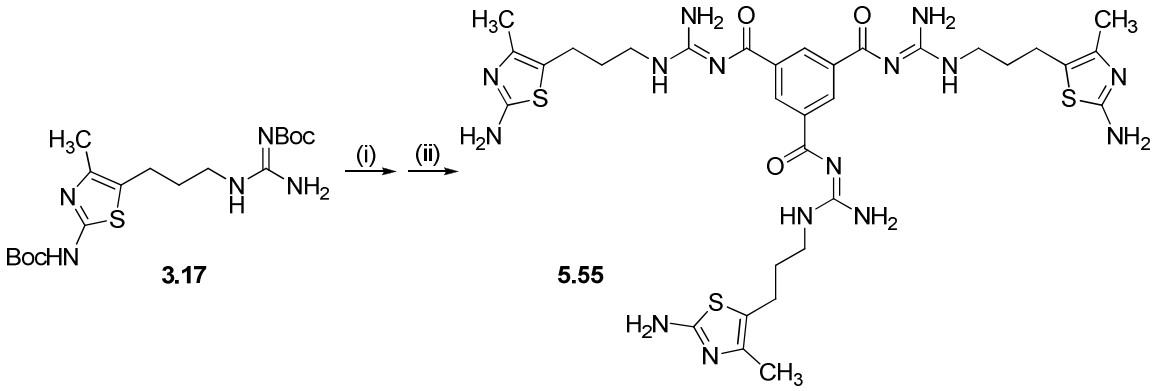


Compd.	X	n[a]	R	Compd.	X	n[a]	R
5.44		4	CH_3	**5.47**		8	CH_3
5.45		6	CH_3	**5.48**		10	CH_3
5.46		3	CH_3	**5.49** (n=1)		8	H
				5.50 (n=3)		12	H

Compd.	X	n[a]	R
5.51		24	CH_3
5.52		9	CH_3
5.53 (n=2)		13	CH_3
5.54 (n=3)		15	CH_3

Scheme 5.4. General procedure for the preparation of bivalent acylguanidines **5.44-5.54**. Reagents and conditions: (i) for **5.44-5.52**: pertinent dicarboxylic acid (1 eq), **3.17** or **3.18** (2 eq), EDAC (2 eq), HOBt (2 eq), DIEA (2 eq), DCM/abs or DMF, 16 h, rt; for **5.53** and **5.54**: pertinent dicarboxylic acid[22] (1 eq), **3.17** (2 eq), EDAC (2.1 eq), DMAP (cat.), DIEA (2.1 eq), DMF, 15 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt. [a] n = number of atoms between the two carbonyl groups.

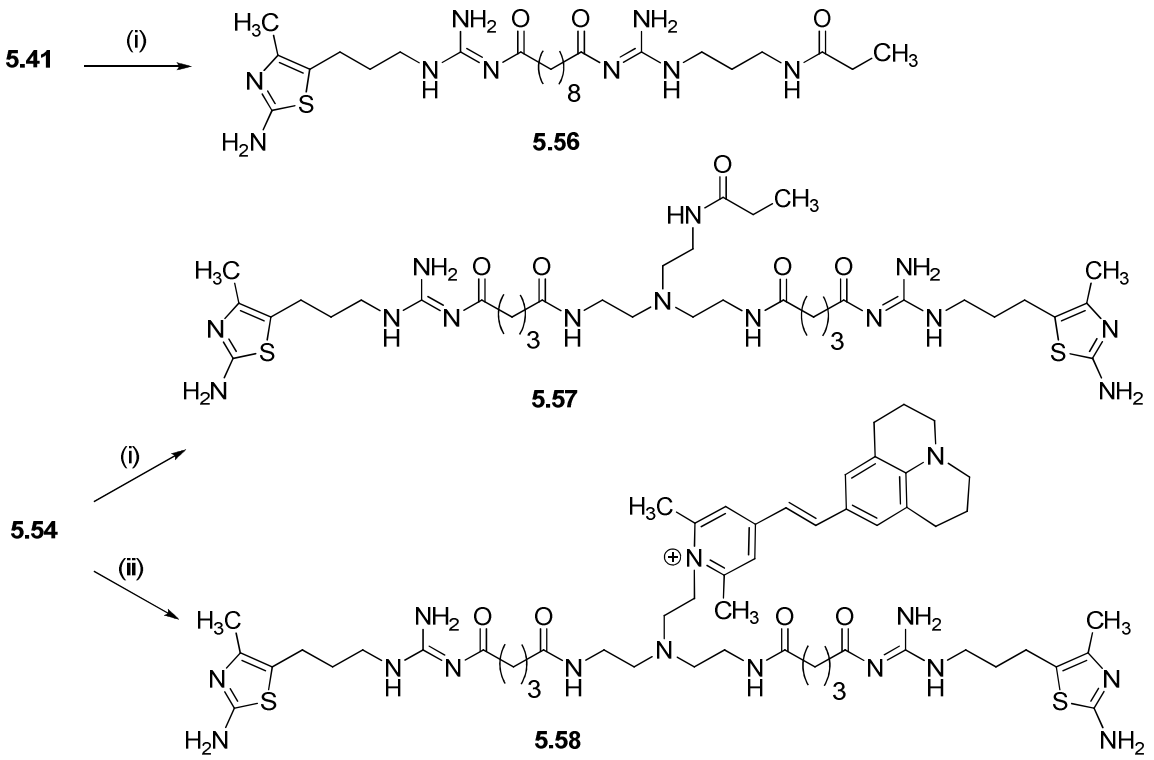
Compound **5.55**, a prototypical ligand containing three 3-(2-amino-4-methylthiazol-5-yl)-propylguanidine moieties was synthesized using three equivalents of the guanidine building block **3.17** and the coupling reagents, respectively, and one equivalent of benzene-1,3,5-tricarboxylic acid (Scheme 5.5). Finally, removal of the protecting groups under acidic conditions gave the "trivalent" compound, which was purified by preparative RP-HPLC.



Scheme 5.5. Synthesis of the trivalent acylguanidine **5.55**. Reagents and conditions: (i) Benzene-1,3,5-tricarboxylic acid (0.33 eq), EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 24 h, rt; (ii) 20 % TFA, DCM/abs, 5 h, rt.

Labeling of bivalent ligands 5.41 and 5.54

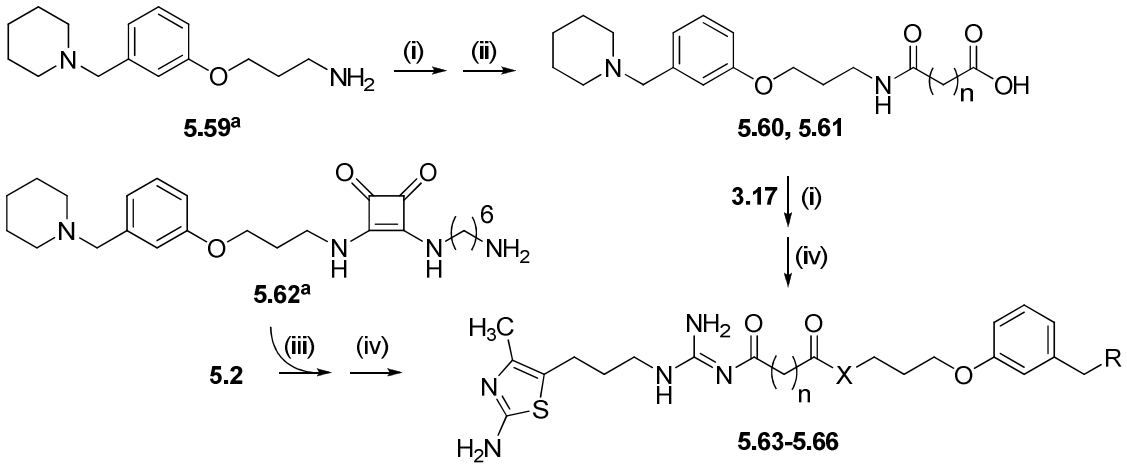
The free amino groups in compounds **5.41** and **5.54** were propionylated by stirring with succinimidyl propionate for a few hours at room temperature affording the compounds **5.56** and **5.57**. These propionamides were prepared and pharmacologically investigated in "cold" form with respect to the optional synthesis of the corresponding bivalent radioligands. The radioactive form of the used succinimidyl ester is a standard reagent for tritium labeling at the last synthetic step. In addition, the fluorescent compound **5.58** was synthesized from **5.54** and the fluorescent pyrylium dye py-1 (for chemical structure see chapter 3, Scheme 3.5) by ring transformation within one hour at room temperature.

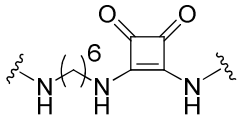


Scheme 5.6. Synthesis of compounds **5.56**-**5.58**. Reactions and conditions: (i) **5.41** or **5.54** (1 eq), succinimidyl propionate (0.8 eq), NEt_3 (3 eq), MeCN, 4-5 h, rt; (ii) **5.54** (2 eq), **py-1**[23] (1 eq), NEt_3 (7.5 eq), MeCN, DMF, 1 h, rt.

Synthesis of heterobivalent compounds by combination of H_2R agonistic and antagonistic moieties

3-[3-(Piperidin-1-ylmethyl)phenoxy]propan-1-amine **5.59**[24] was converted into the amides **5.60** and **5.61** by acylation with **5.1** and hexadecanedioic acid, respectively, using EDAC, HOBt and DIEA as coupling reagents. Removal of the benzyl protecting group (**5.60**) resulted in a by-product lacking the piperidino group. This cleavage product could not be separated until purification by flash chromatography after the next coupling step. According to Scheme 5.7, coupling of the dicarboxylic acid mono-amides **3.60** and **3.61** with **3.17** and coupling of the recently synthesized squaramide derivative **5.62**[25] with **5.2** yielded the Boc-protected hybrid molecules **5.63a-5.66a**. The protecting groups were removed in a few hours by treating with TFA in DCM (TLC control) to obtain the heterobivalent acylguanidines **5.63-5.66**, which were purified by preparative RP-HPLC.



Compd.	n	Compd.	n	X	R	Compd.	n	X	R
5.60	8	**5.63**	8	NH	Piperidine				
5.61	14	**5.64**	14	NH	Piperidine				
		5.65	8	NH	H	**5.66**	8		Piperidine

Scheme 5.7. General procedure for the preparation of bivalent acylguanidines **5.63-5.66**. Reagents and conditions: (i) **5.1**, hexadecanedioic acid or **3.17** (1 eq), EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 16 h, rt; (ii) for **5.60**: H_2, Pd/C (10 %), MeOH, 3 h, rt; (iii) **5.62**[25] (1 eq), CDI (1.6 eq), **5.2** (1.5 eq), THF/abs, DMF, 14 h, rt; (iv) 20 % TFA, DCM/abs, 3-5 h, rt. [a] Compounds **5.59** and **5.62** were provided by Dr. D. Erdmann. For experimental data see Ref.[25]

5.3 Pharmacological results and discussion

All synthesized compounds were examined for H_2R agonism in a membrane steady-state GTPase assay at human (h) and guinea pig (gp) H_2R-$Gs\alpha_S$ fusion proteins expressed in Sf9 insect cells (Tables 5.1-5.3).[26] With respect to information about the molecular determinants of different agonist potencies at human and guinea pig H_2R orthologs, selected bivalent ligands were tested on H_2R mutants, in which Cys-17 and Ala-271 in the hH_2R were replaced by Tyr-17 and Asp-271 as in the gpH_2R and four different amino acids in the e2 loop were reciprocally mutated (hH_2R-C17Y-A271D-$Gs\alpha_S$, hH_2R-C17Y-$Gs\alpha_S$, hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$) (Table 5.4).[26-28] Moreover, to verify the histamine receptor subtype selectivity, representative compounds were investigated in GTPase assays using recombinant human histamine H_1, H_3 and H_4 receptors (Table 5.5).

5.3.1 Histamine H_2 receptor agonism at human and guinea pig H_2R fusion proteins in the GTPase assay

Unsymmetrical bivalent ligands (Table 5.1)

To elaborate the role and interaction site of the second pharmacophoric moiety, unsymmetrical compounds bearing two different acylguanidine moieties were investigated. Based on the highly potent N^1,N^{10}-bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (**4.27**), structural modifications were focused on the eastern part of the molecule.

Except for compound **5.27**, which showed full agonism at gpH_2R-Gsα_S, all synthesized unsymmetrical bivalent "bis-acylguanidines" (**5.26-5.41** and **5.56**) proved to be moderate to potent partial agonists at hH_2R-Gsα_S and gpH_2R-Gsα_S fusion proteins. The most potent compounds of this series surpassed the potency of histamine about 170 and 2500 times at hH_2R-Gsα_S and gpH_2R-Gsα_S, respectively. In agreement with results obtained for symmetrical compounds (cf. chapter 4), the removal of one 4-methyl group at the aminothiazole ring (**5.26** *vs.* **4.27**) or the exchange of the 2-amino-4-methylthiazole by an imidazole ring (**5.27** *vs.* **4.27**) led to slightly decreased potencies, but increased efficacies at both receptors. Interestingly, the potencies of **5.26** and **5.27** at both receptors were always between the potencies of the symmetrical analogs (compare **5.26** with **4.11** and **4.27**, **5.27** with **4.27** and **4.31**, Fig. 5.2). In contrast, the efficacies were close to the high efficacies of the corresponding "bis-imidazole" **4.31** and "bis-aminothiazole" **4.11**, respectively (cf. Figure 5.2). This also holds for **5.28**, the combination of a 2-amino-4-methylthiazolylpropylguanidine with the weakly potent 1,2,4-triazolylpropylguanidine (compare **5.28** with **4.24** and **4.27**). In conclusion, both heterocycles of the unsymmetrical compounds nearly additively contribute to potency, whereas efficacy seems to be determined by the "more efficacious moiety".

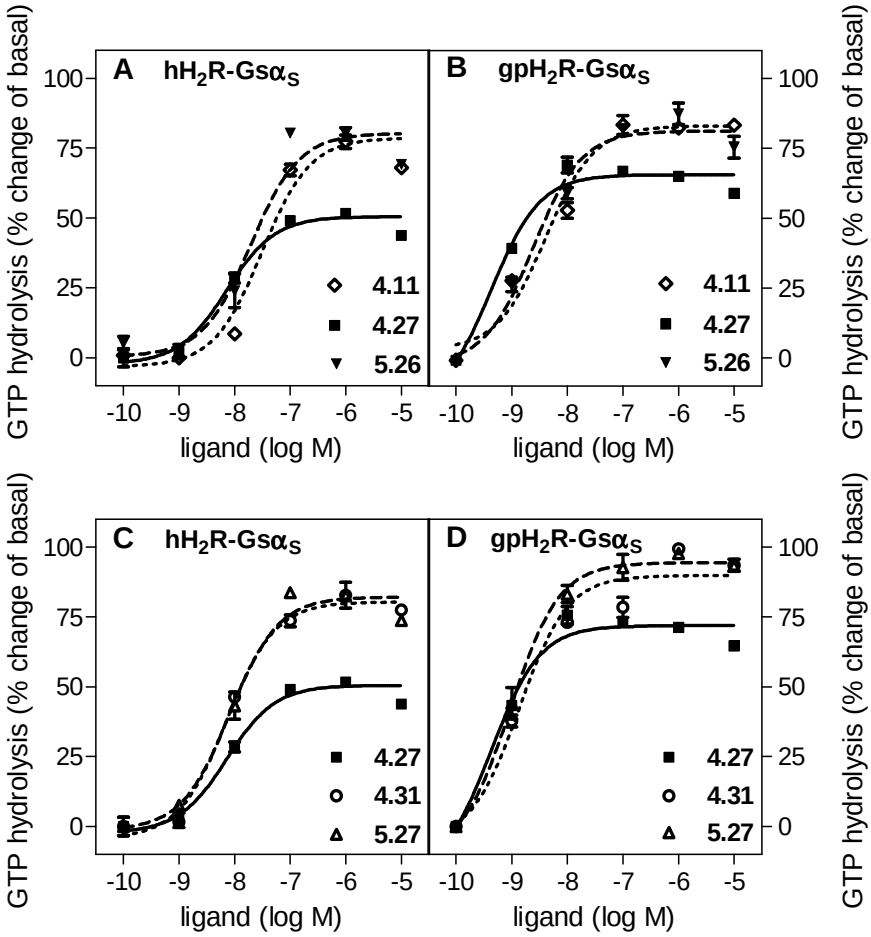


Figure 5.2: Histamine H_2 receptor agonism of the unsymmetrical bivalent ligand **5.26** compared to the symmetrical bivalent ligands **4.11** and **4.27** at membranes expressing hH_2R-$Gs\alpha_S$ (**A**) and gpH_2R-$Gs\alpha_S$ (**B**) and H_2R agonism of the unsymmetrical bivalent ligand **5.27** compared to the symmetrical bivalent ligands **4.27** and **4.31** at hH_2R-$Gs\alpha_S$ (**C**) and gpH_2R-$Gs\alpha_S$ (**D**). Data of representative experiments performed in duplicate, expressed as percentage change in GTPase activity relative to the maximum effect induced by histamine (100 µM).

Replacing one hetaryl group of **4.27** with a phenyl ring (**5.29**) resulted in a drop of potency by one to almost two orders of magnitude, whereas efficacy was not affected. Whereas methyl substitution at γ-position of the side chain was well tolerated (**5.29** *vs.* **5.30**), an additional phenyl ring, resulting in a space filling diphenylpropyl residue (**5.31**), further decreased the potency at both receptors. As a result of replacing phenyl with cyclohexyl (**5.29** *vs.* **5.32**), agonistic potency was further decreased by 3- and 14-fold at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Shortening the carbon chain between the guanidino group and the phenyl ring from three to one methylene groups (**5.33**) resulted in moderately increased potency. The methyl-branched analogs of **5.33** (**5.34**, **5.35**) are

chiral compounds. Very recently, monovalent chiral N^G-acylated hetarylpropylguanidines did not show significant stereoselectivity.[29] This also holds for bivalent acylguanidines (**3.34** *vs.* **3.35**): only marginally higher potencies (eudismic ratio of about 2) resided in the (*S*)-enantiomer. Moreover, mono- (**5.36**) or di- (**5.37**) substitution of the phenyl ring with electron releasing methoxy groups had no significant effect on the agonistic potency. Interestingly, efficacies were not affected by these minor structural variations (compare **5.29**-**5.37**). Thus, in agreement with the results obtained for **5.26**-**5.28**, both acylguanidine moieties of the unsymmetrical compounds contribute to potency, whereas efficacy seems to be determined by the more efficacious moiety.

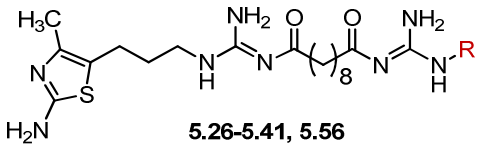
Replacement of one (het)arylalkylguanidine with small alkylguanidine moieties afforded rather potent H_2R agonists with EC_{50} values in the low nanomolar range at both hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$. Herein, methyl- (**5.38**) and isobutyl- (**5.40**) were superior to the corresponding propylguanidine (**5.39**). The introduction of an additional primary amino group at the propyl chain led to slightly increased potencies and efficacies (**5.39** *vs.* **5.41**). Notably, the free amino group allowed for the attachment of radio labels. The conversion of the amine (**5.41**) to the propionamide **5.56** resulted in moderately (up to a factor of 2.7) decreased potencies at both receptors. However, the "cold" potential bivalent radioligand **5.56** revealed EC_{50} values of 56 nM and 9.5 nM at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Presumably, the affinity of this compound is sufficiently high to use the corresponding "hot" form in investigations on the ligand-receptor stoichiometry compared to monomeric radioligands.

Replacing the second basic acylguanidino group of **4.27** with a simple amide group caused a 7- and even 60-fold decrease in potency at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively (**4.27** *vs.* **5.42**), corroborating the importance of a basic centre at an appropriate distance to the pharmacophore to obtain highly potent bivalent H_2R agonists. This is in accordance with the results obtained for monovalent aminothiazolylpropyl-guanidines containing primary amino groups in the alkanoyl side chain (cf. chapter 3).

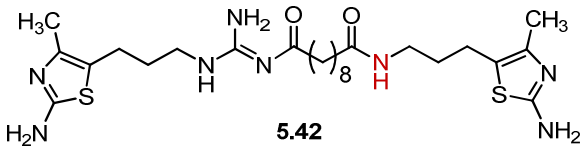
Furthermore, in agreement with the results obtained for symmetrical compounds, linkage of a 3-(2-amino-4-methylthiazolyl)propylguanidine with an imidazolylpropylguanidine by a very flexible 20-membered carbon chain, a length predicted to be optimal to bridge two neighboring receptors, resulted in a drastic decrease in potency at the gpH_2R-$Gs\alpha_S$ and a complete loss of agonistic activity at the hH_2R-$Gs\alpha_S$ (compare **5.43** with **4.19** and

4.30). To some extent this may depend on an entropic cost caused by fixing the highly flexible molecule on the receptor surface, as the Gibbs free energy released upon ligand binding results from enthalpic and entropic contributions ($\Delta G = \Delta H - T\Delta S$). However, these results argue against the occupation of two neighboring receptors.

Table 5.1. Agonist potencies and efficacies of unsymmetrical acylguanidines and reference compounds at hH_2R-Gsα_S and gpH_2R-Gsα_S fusion proteins in the steady-state GTPase assay.[a]



5.26-5.41, 5.56



5.42

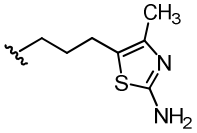
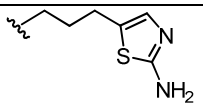
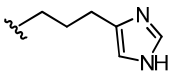
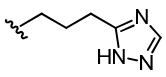
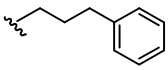
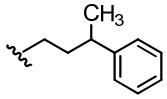
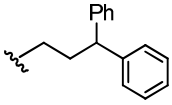
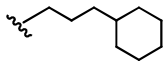
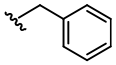
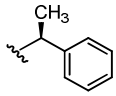
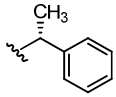
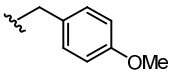
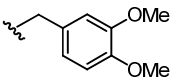
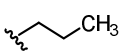
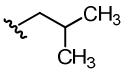
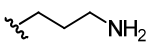
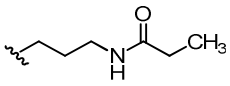
No.	R	hH_2R-Gsα_S			gpH_2R-Gsα_S			**EC_{50} (hH_2R-Gsα_S) / EC_{50} (gpH_2R-Gsα_S)**
		pEC_{50} ± SEM	**E_{max} ± SEM**	**Pot_{rel}**	**pEC_{50} ± SEM**	**E_{max} ± SEM**	**Pot_{rel}**	
His[26]	-	5.90 ± 0.09	1.00	1.0	5.92 ± 0.09	1.00	1.0	1.05
4.11[b]	-	7.67 ± 0.07	0.75 ± 0.03	58.9	8.30 ± 0.22	0.94 ± 0.01	239.9	4.27
4.27		8.11 ± 0.25	0.53 ± 0.04	162.2	9.41 ± 0.15	0.79 ± 0.07	3,090	19.90
4.31[b]	-	8.21 ± 0.07	0.81 ± 0.02	204.2	8.94 ± 0.16	0.98 ± 0.05	1,047	5.36
5.26		7.86 ± 0.11	0.75 ± 0.04	91.2	8.46 ± 0.30	0.89 ± 0.04	346.7	3.98
5.27		8.12 ± 0.04	0.76 ± 0.05	166.0	9.29 ± 0.10	1.01 ± 0.03	2,344	14.79
5.28		7.40 ± 0.20	0.50 ± 0.04	31.6	7.90 ± 0.08	0.88 ± 0.05	95.5	3.16
5.29		7.16 ± 0.20	0.44 ± 0.05	18.2	7.69 ± 0.25	0.76 ± 0.06	58.9	3.39
5.30		7.19 ± 0.11	0.44 ± 0.02	19.5	7.72 ± 0.03	0.82 ± 0.06	63.1	3.39
5.31		6.81 ± 0.11	0.45 ± 0.11	8.1	7.32 ± 0.12	0.77 ± 0.05	25.1	3.24

Table 5.1. (continued)

5.32		6.72 ± 0.16	0.32 ± 0.02	6.6	6.55 ± 0.01	0.77 ± 0.00	4.3	0.68
5.33		7.66 ± 0.06	0.46 ± 0.03	57.5	8.05 ± 0.05	0.79 ± 0.04	134.9	2.45
5.34		7.68 ± 0.10	0.45 ± 0.02	60.3	8.13 ± 0.26	0.74 ± 0.07	162.2	2.82
5.35		7.38 ± 0.06	0.39 ± 0.02	30.2	7.77 ± 0.32	0.76 ± 0.01	70.8	2.45
5.36		7.56 ± 0.04	0.45 ± 0.03	45.7	7.79 ± 0.08	0.85 ± 0.02	74.1	1.70
5.37		7.56 ± 0.11	0.33 ± 0.01	45.7	7.92 ± 0.16	0.71 ± 0.03	100.0	2.29
5.38		7.91 ± 0.09	0.62 ± 0.03	102.3	8.70 ± 0.05	0.89 ± 0.04	602.6	6.17
5.39		7.50 ± 0.04	0.53 ± 0.02	39.8	8.03 ± 0.21	0.86 ± 0.04	128.8	3.39
5.40		7.96 ± 0.14	0.51 ± 0.02	114.8	8.67 ± 0.02	0.87 ± 0.11	562.3	5.13
5.41		7.68 ± 0.11	0.58 ± 0.03	60.3	8.15 ± 0.10	0.94 ± 0.04	169.8	2.95
5.56		7.25 ± 0.04	0.68 ± 0.11	22.4	8.02 ± 0.01	0.91 ± 0.01	125.9	5.89
5.42	-	7.25 ± 0.21	0.82 ± 0.07	22.4	7.62 ± 0.08	0.96 ± 0.03	50.1	2.34
5.43[c]	-	(-	-	-)[d]	6.54 ± 0.04	0.19 ± 0.07	4.2	-

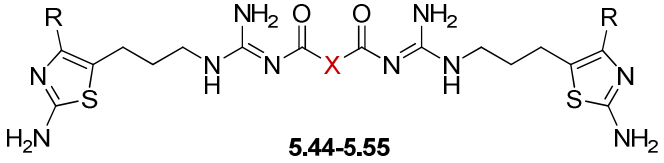
[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R-Gsα_S and gpH_2R-Gsα_S was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 0.1 nM to 10 μM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 2.5 pmol·mg^{-1}·min^{-1} and activities stimulated by histamine (100 μM) ranged between ≈ 2 and 13 pmol·mg^{-1}·min^{-1}. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of 2-5 independent experiments performed in duplicate. The relative potency of histamine was set to 1.0, and the potencies of other agonists were referred to this value. [b] For chemical structure see chapter 4. [c] For chemical structure see Scheme 5.3. [d] No agonistic activity.

Structural modifications of the spacer (Table 5.2)

The chemical structure of the spacer plays a critical role with respect to the spatial orientation of the pharmacophoric groups at the receptor of interest. In addition, the linker significantly contributes to lipophilicity and flexibility and affects the overall profile of bivalent ligands including drug-like properties and interactions with off-targets. It is not possible to draw up generally valid rules for the prediction of the best suited spacer. To date, most bivalent ligand approaches are based on the use of flexible linkers with well-balanced hydrophilic and lipophilic properties.[2,30-31] Consequently, after the investigation of homobivalent ligands with typically used hydrophobic methylenic linkers (cf. chapter 4), we incorporated different functional groups such as disulfide, ether and amide, phenylene groups as well as branched linkers, to evaluate bivalent acylguanidines with increased hydrophilicity as well as conformationally more constrained compounds.

With exception of compounds **5.49** and **5.50**, which showed moderate potencies, all structural modifications of the spacer (**5.44-5.54**) considerably decreased the agonistic potency at $hH_2R\text{-}Gs\alpha_S$ ($pEC_{50} \leq 6.8$) and $gpH_2R\text{-}Gs\alpha_S$ ($pEC_{50} \leq 7.7$), respectively, compared to the analogs containing methylenic linkers. As expected,[31] potencies and efficacies at the H_2Rs dramatically decreased in case of the more constrained compounds **5.44-5.47**, emphasizing the importance of spacer flexibility. It should be taken into account that purely methylenic spacers possibly impair solubility and tend to enhance binding to membranes. Therefore, incorporation of hydrophilic groups seemed reasonable to significantly reduce the $logD_{[7.4]}$ values of the compounds (cf. Table 8.2). Interestingly, **5.48-5.54** with inserted hydrophilic units showed decreased potencies, whereas efficacies were significantly increased at both receptors. Compounds **5.48**, **5.49** and **5.52** were full agonists at the $gpH_2R\text{-}Gs\alpha_S$. Actually, in contrast to compounds with a 20-membered alkanediyl spacer (**4.13**, **4.19**, **4.30**, **5.43**), which revealed antagonistic activities at the $hH_2R\text{-}Gs\alpha_S$, elongation of the hydrophilic spacer up to 24 atoms resulted in compounds with retained weak H_2R agonistic activity (**5.51**: $hH_2R\text{-}Gs\alpha_S$, $pEC_{50} = 6.20$, $E_{max} = 0.45$). Notably, in bivalent ligands with inserted disulfide group, a 12-membered chain connecting the carbonyl groups (**5.50**) turned out to be superior to the (supposedly optimal) 8-membered chain (**5.49**). The corresponding monovalent analog **3.56** was about 10-fold less potent, whereas the acetic acid thioester of **3.65** (**3.55**) was equipotent with **5.50**.

Table 5.2. Agonistic potencies and efficacies of bivalent aminothiazolylpropylguanidines **5.44-5.55** and reference compounds at hH_2R-Gsα_S and gpH_2R-Gsα_S fusion proteins in the steady-state GTPase assay.[a]



5.44-5.55

	R
4.25-4.30, 5.44-5.48, 5.51-5.55	CH_3
5.49, 5.50	H

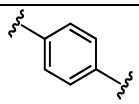
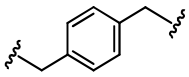
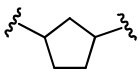
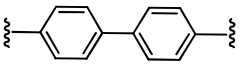
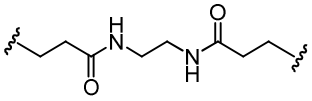
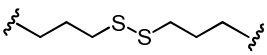
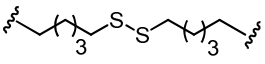
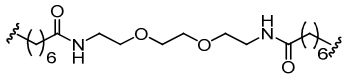
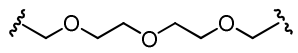
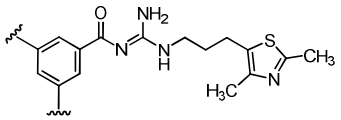
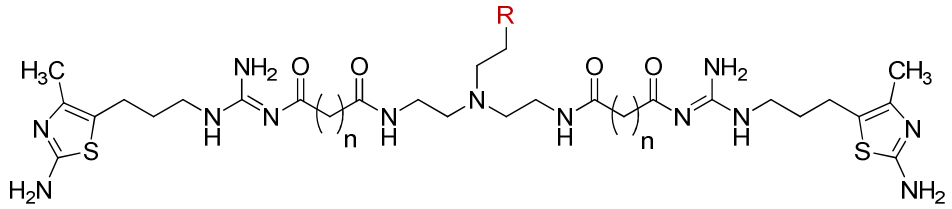
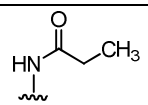
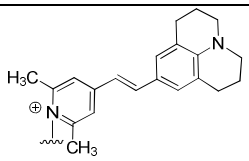
No.	X	hH_2R-Gsα_S			gpH_2R-Gsα_S			EC_{50} hH_2R / EC_{50} gpH_2R
		pEC_{50} ± SEM	E_{max} ± SEM	Pot_{rel}	pEC_{50} ± SEM	E_{max} ± SEM	Pot_{rel}	
His[26]	-	5.90 ± 0.09	1.00	1.0	5.92 ± 0.09	1.00	1.0	1.05
4.25	$(CH_2)_4$	7.24 ± 0.22	0.68 ± 0.03	21.9	8.59 ± 0.30	0.90 ± 0.05	467.7	22.39
4.27	$(CH_2)_8$	8.11 ± 0.25	0.53 ± 0.04	162.2	9.41 ± 0.15	0.79 ± 0.07	3090.3	19.90
4.28	$(CH_2)_{10}$	7.78 ± 0.17	0.46 ± 0.04	75.9	8.57 ± 0.32	0.66 ± 0.05	446.7	6.17
4.29	$(CH_2)_{14}$	7.59 ± 0.22	0.12 ± 0.02	49.0	7.46 ± 0.01	0.51 ± 0.02	102.3	0.78
4.30	$(CH_2)_{20}$	(-	-	-)[b]	6.48 ± 0.37	0.58 ± 0.02	3.6	-
5.44		5.46 ± 0.17	0.13 ± 0.01	0.4	6.08 ± 0.08	0.73 ± 0.03	1.5	4.17
5.45		6.62 ± 0.23	0.79 ± 0.07	5.3	7.86 ± 0.06	0.83 ± 0.17	87.1	17.38
5.46		6.82 ± 0.01	0.69 ± 0.11	8.3	7.22 ± 0.21	0.47 ± 0.02	12.0	2.51
5.47		6.78 ± 0.06	0.16 ± 0.02	7.6	7.07 ± 0.12	0.63 ± 0.03	14.1	1.95
5.48		6.25 ± 0.18	0.64 ± 0.04	2.2	6.55 ± 0.10	1.05 ± 0.10	4.3	2.00
5.49		7.13 ± 0.22	0.73 ± 0.06	17.0	7.69 ± 0.04	1.00 ± 0.02	58.9	3.63
5.50		7.48 ± 0.06	0.45 ± 0.08	38.0	7.95 ± 0.10	0.70 ± 0.03	107.2	2.95

Table 5.2. (continued)

5.51		6.17 ± 0.26	0.45 ± 0.11	1.9	6.64 ± 0.24	0.22 ± 0.02	5.3	2.95
5.52		6.71 ± 0.19	0.58 ± 0.11	6.5	6.91 ± 0.05	1.03 ± 0.04	9.8	1.58
5.55		5.88	0.07	1.0	6.02	0.09	1.3	1.38



	n	**R**							
5.53	2	NH_2	6.28 ± 0.07	0.61 ± 0.01	2.4	7.27 ± 0.04	0.76 ± 0.03	22.4	9.77
5.54	3	NH_2	6.48 ± 0.01	0.59 ± 0.01	3.8	7.71 ± 0.01	0.93 ± 0.01	61.7	16.98
5.57	3		6.10 ± 0.10	0.54 ± 0.02	1.6	7.15 ± 0.05	0.86 ± 0.02	17.0	11.22
5.58	3		6.75	0.21	7.1	6.93	0.65	10.2	1.51

[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R-Gsα_S and gpH_2R-Gsα_S was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 0.1 nM to 10 μM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 2.5 $pmol \cdot mg^{-1} \cdot min^{-1}$, and activities stimulated by histamine (100 μM) ranged between ≈ 2 and 13 $pmol \cdot mg^{-1} \cdot min^{-1}$. The efficacy ($E_{max}$) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of 1-6 independent experiments performed in duplicate. The relative potency of histamine was set to 1.0, and the potencies of other agonists were referred to this value. [b] No agonistic activity.

Despite the weak H_2R agonistic potency of **5.44-5.54**, the successful preparation of bivalent ligands with branched linkers (**5.53** and **5.54**) is especially worth mentioning. The branched linkers allowed for the attachment of fluorescence and radio labels without affecting the pharmacophoric hetarylpropylguanidine moieties. However, the conversion of the basic amine in **5.54** to the non-basic propionamide group in **5.57** resulted in a

significant decrease in potency (pEC_{50} (hH_2R-$Gs\alpha_S$) = 6.48 → 6.10; pEC_{50} (gpH_2R-$Gs\alpha_S$) = 7.71 → 7.15), whereas efficacy was not affected. The positively charged fluorescent pyridinium compound **5.58** was slightly more potent than **5.54** at hH_2R-$Gs\alpha_S$ (pEC_{50} = 6.75), but considerably less potent at gpH_2R-$Gs\alpha_S$ (pEC_{50} = 6.93), and efficacies were reduced at both receptors.

Finally, the prototypical "trivalent" compound **5.55** with three aminothiazolyl-propylguanidine portions and a constrained aromatic spacer was devoid of (noteworthy) agonistic activity at both hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Thus, the introduction of an additional pharmacophoric moiety proved to be inappropriate to improve the H_2R agonistic activity.

Contribution of the second pharmacophoric moiety to H_2R agonistic activity

The structure-activity relationships of bivalent H_2R agonistic acylguanidines are not compatible with the concept that such ligands are capable of "bridging" the orthosteric recognition sites of receptor dimers, as highest potency resided in compounds with insufficient spacer length. Figure 5.3 gives an overview of the agonistic potencies of the title compounds as determined in the GTPase assay. Most strikingly, the combination of two hetarylpropylguanidine pharmacophores with decanedioyl spacer resulted in the most potent H_2R agonists known to date.

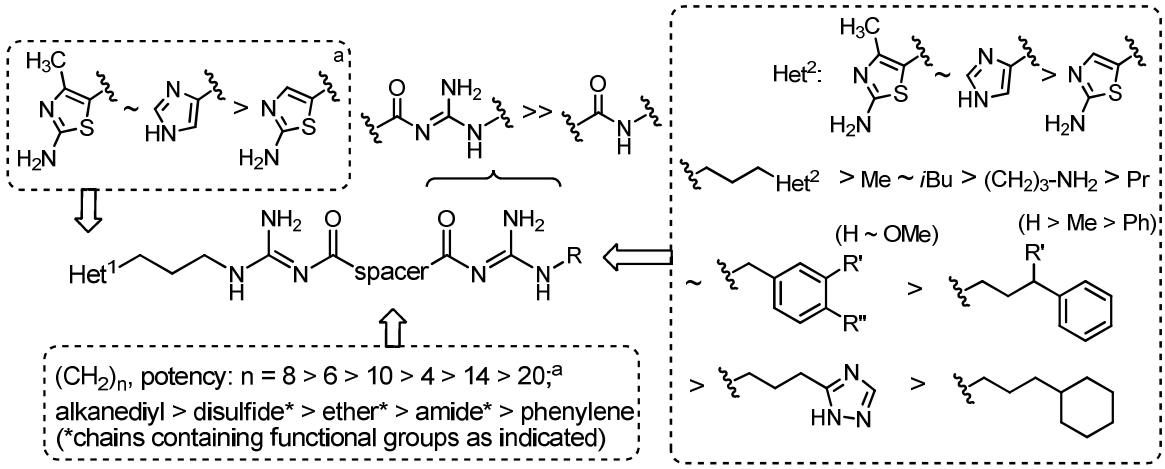


Figure 5.3. H_2R agonistic potency (GTPase assay) of bivalent acylguanidines: overview of structure-activity relationships. [a] cf. twin compounds in chapter 4.

To estimate the contribution of the second pharmacophore to H_2R agonistic activity and to factor out the contribution of the spacer itself, the bivalent acylguanidine-type ligands should be compared with appropriate monovalent counterparts. Most monovalent

compounds can only be considered an approximation to a monomeric analog as alkyl chains and various functional groups (carboxy, amine, amide, phenyl or cyclohexyl) may also confer to H_2R activity. Actually, the choice of an appropriate monomeric counterpart is very tenuous. The pentanoylguanidine **3.20** corresponds to the bisected compound **4.27** and therefore was considered the best possible monomeric counterpart of the highly potent bivalent H_2R agonist **4.27**. As determined in the GTPase assay, the twin compound **4.27** was up to two orders of magnitude superior to its monovalent counterpart **3.20** (hH_2R-$Gs\alpha_S$, pEC_{50}, 8.11 → 7.06; gpH_2R-$Gs\alpha_S$, pEC_{50}, 9.41 → 7.54). Moreover, comparison of the H_2R agonistic potency of **4.27** with **5.38**, **5.42**, **3.20**, **3.21** and **3.31** (cf. Figure 5.4) indicated that the contribution of the spacer to H_2R agonistic activity is rather low and the second guanidine moiety is not merely an additional cationic head. Thus, both guanidino groups are involved in specific interactions with the H_2R. These results are consistent with the presence of an accessory binding site at the H_2R. Depending on the substitution pattern, the acylguanidine moiety seems to be a versatile structural motif to address an accessory recognition site at the H_2R. Ionic interactions between the positively charged acylguanidine group and negatively charged amino acid residues are conceivable. This bears a formal resemblance to the message-address concept proposed by Schwyzer.[32] The hetarylpropylguanidine pharmacophore acts as the message component that is recognized by the receptor and the cationic address recognizes a unique subsite and provides additional binding affinity.

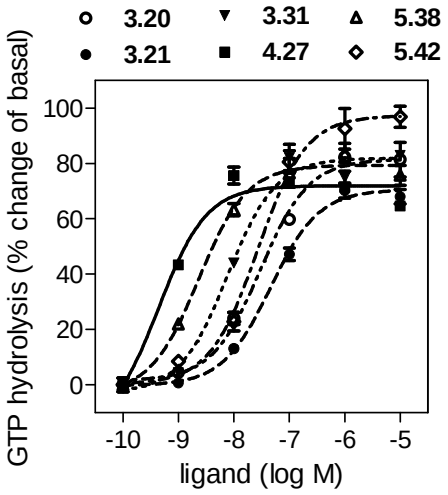


Figure 5.4. Histamine H_2R agonism of **3.20**, **3.21**, **3.31**, **4.27**, **5.38** and **5.42** in membranes expressing the gpH_2R-$Gs\alpha_S$. Data of representative experiments, expressed as percentage change in GTPase activity relative to the maximum effect induced by histamine (100 µM). For exact chemical structures and pEC_{50} values of **3.20**, **3.21** and **3.31** see chapter 3.

Heterobivalent compounds with combined H_2R agonistic and antagonistic moieties (Table 5.3)

For most bivalent ligands interacting with two binding sites (orthosteric-orthosteric or orthosteric-accessory), co-activation *via* two agonistic pharmacophores is required for maximal effects.[33-35] However, these findings are in contrast to several other reports in which maximal signaling results from the combination of agonistic with antagonistic or inverse agonistic pharmacophores.[36-37] In this context, heterobivalent ligands with combined agonistic and antagonistic functionalities, i.e. agonistic/antagonistic hybrid molecules, were synthesized and investigated regarding their H_2R activities. The hybrid molecules were constructed by combining the agonistic 3-(2-amino-4-methylthiazolyl)-propylguanidine moiety with the core structure of piperidinomethylphenoxyalkylamine-type antagonists (Figure 5.5).

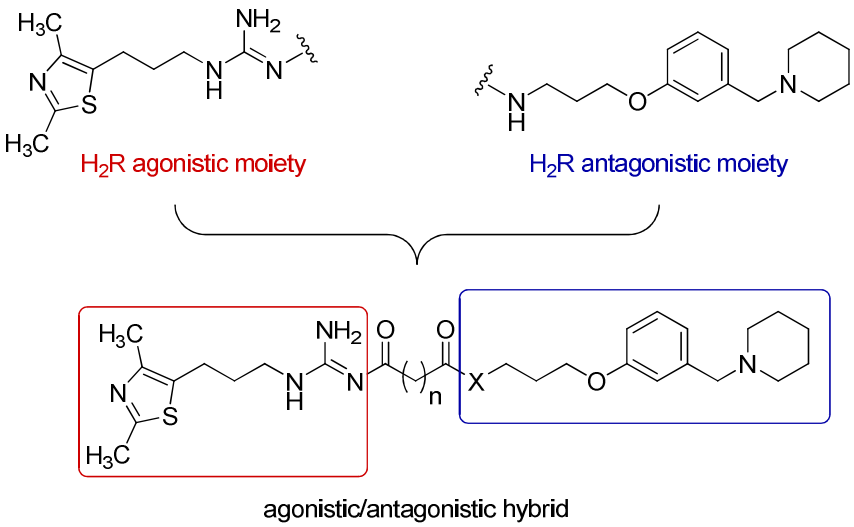


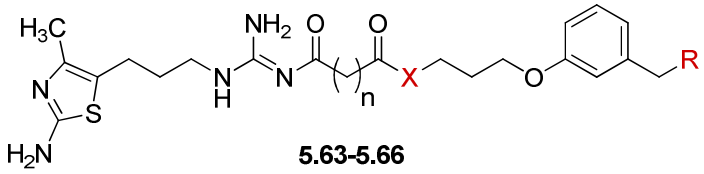
Figure 5.5. Design of H_2R agonistic/antagonistic hybrid molecules. The agonistic building block derived from N^G-acylated 3-(2-amino-4-methylthiazol-5-yl)propylguanidines; the antagonistic building block derived from roxatidine-related piperidinomethylphenoxypropylamine derivatives.

The hybrid molecule **5.63** showed moderate partial agonism at hH_2R-$Gs\alpha_S$ (pEC_{50} = 7.32), but potent neutral antagonism at the gpH_2R-$Gs\alpha_S$ (pK_B = 7.91). As expected, removal of the basic piperidino group in the antagonistic roxatidine-related pharmacophore (**5.65**) resulted in a shift from antagonistic to agonistic activity at the gpH_2R-$Gs\alpha_S$, i.e. **5.65** was a weak agonist at both receptors. Notably, elongation of the spacer combining the agonistic and the antagonistic pharmacophores (n: 8 → 14, cf. **5.63** *vs.* **5.64**) resulted in the opposing biological responses. In contrast to **5.63**, **5.64** is a

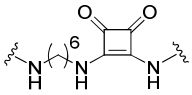
neutral antagonist at the hH_2R-$Gs\alpha_S$ (pK_B = 6.49) and moderate partial agonist at the gpH_2R-$Gs\alpha_S$ (pEC_{50} = 7.15). Moreover, the introduction of a squaramide group, which was recently found to increase both the H_2R antagonistic activities and the preference for the H_2R over the H_3R,[25] had no significant effect on the H_2R mediated response (compare **5.64** with **5.66**).

With respect to H_2R agonistic potency, the hybrid approach combining H_2R agonistic and H_2R antagonistic moieties proved to be inappropriate. However, the discrepancies between the qualities of action depending of the H_2R species orthologs give rise to speculations about different binding modes of these hybrid molecules at the gpH_2R compared to the hH_2R.

Table 5.3. Agonistic and antagonistic effects of agonistic/antagonistic hybrid molecules at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ fusion proteins in the steady-state GTPase assay.[a]



5.63-5.66

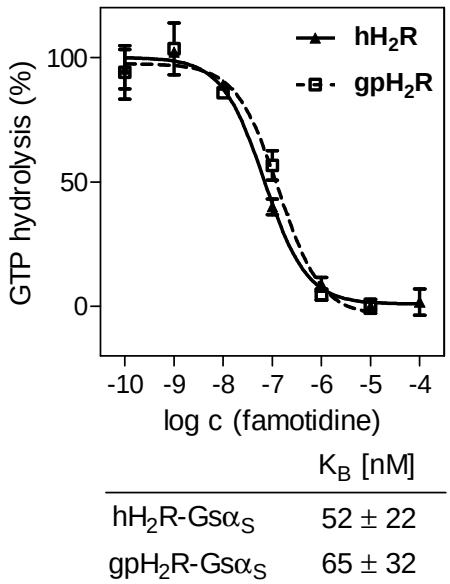
No.	n	X	R	hH_2R-$Gs\alpha_S$			gpH_2R-$Gs\alpha_S$		
				$pEC_{50}/(pK_B)$ ±SEM	E_{max} ±SEM	Pot_{rel}	$pEC_{50}/(pK_B)$ ±SEM	E_{max} ±SEM	Pot_{rel}
His				5.90 ± 0.09	1.00	1.0	5.92 ± 0.09	1.00	100
5.63	8	NH		7.32 ± 0.06	0.60 ± 0.03	26.3	(7.91 ± 0.02)	-	-
5.64	14	NH		(6.49 ± 0.19)	-	-	7.15 ± 0.05	0.86 ± 0.02	17.0
5.65	8	NH	H	6.82 ± 0.14	0.57 ± 0.04	8.3	7.05 ± 0.06	0.81 ± 0.02	13.5
5.66	8			(7.03 ± 0.10)	-	-	6.33 ± 0.07	0.21 ± 0.03	2.6

[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM as appropriate to generate saturated concentration-response curves. For antagonism, reaction mixtures contained histamine (1 µM) and ligands at concentrations from 1nM to 100 µM. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 2.5 $pmol \cdot mg^{-1} \cdot min^{-1}$ and activities stimulated by histamine (100 µM) ranged between ≈ 2 and 13 $pmol \cdot mg^{-1} \cdot min^{-1}$. The efficacy ($E_{max}$) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data

shown are means ± SEM of 2-8 independent experiments performed in duplicate. The relative potency of histamine was set to 1.0, and the potencies of other agonists were referred to this value. IC_{50} values were converted to K_B values using the Cheng-Prusoff equation.[38]

Inhibition of the 5.26-stimulated GTP hydrolysis by famotidine

According to the procedure described in chapter 4, the H_2R-mediated effect of bivalent acylguanidine-type ligands was confirmed by the investigation of representative unsymmetrical bivalent ligands in the presence of the H_2R antagonist famotidine. As an example, the inhibition of the **5.26**-stimulated GTPase activity by the H_2R antagonist is depicted in Figure 5.6. At both, hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, **5.26**-stimulated GTP hydrolysis was inhibited in a concentration-dependent manner, confirming the measured GTPase activity to be stimulated via the H_2R. Thus, direct G-protein activation can be clearly ruled out. The calculated K_B values of famotidine (52 ± 22 nM and 65 ± 32 nM, Fig. 5.6) determined against **5.26** at hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$ respectively, are comparable to data obtained from GTPase assays using histamine as the H_2R agonist (reported K_B values, hH_2R-$Gs\alpha_S$: 48 ± 10 nM, gpH_2R-$Gs\alpha_S$: 38 ± 3 nM).[39]



	K_B [nM]
hH_2R-$Gs\alpha_S$	52 ± 22
gpH_2R-$Gs\alpha_S$	65 ± 32

Figure 5.6. Concentration-dependent inhibition of GTP hydrolysis by famotidine using **5.26** as agonist at concentrations of 10 nM and 1 nM at the hH_2R-$Gs\alpha_S$ (solid line) and the gpH_2R-$Gs\alpha_S$ fusion proteins (dashed line), respectively. Data points are means of a representative experiment performed in duplicate. IC_{50} values were converted to K_B values using Cheng-Prusoff equation.[38]

5.3.2 Agonistic activity on histamine H_2R mutants/chimera

In agreement with previous studies,[26,40-42] all newly synthesized bivalent acylguanidines (except **5.32** and **5.63**) were significantly more potent and efficacious at the gpH_2R relative to the hH_2R, as revealed in GTPase assays (cf. Tables 5.1-5.3). As discussed in chapter 4, these differences may result from species-dependent interactions with both the

orthosteric and the putative accessory binding site. The latter is probably located in the extracellular domain, and amino acids in the e2 loop are possible candidates to interact with bivalent ligands. To study the role of particular amino acids and the e2 loop, selected bivalent ligands were tested on H_2R mutants, in which Cys-17 and Ala-271 in the hH_2R were replaced by Tyr-17 and Asp-271 as in the gpH_2R and four different amino acids in the e2 loop were reciprocally mutated (hH_2R-C17Y-A271D- $Gs\alpha_S$, hH_2R-C17Y- $Gs\alpha_S$, hH_2R-gpE2- $Gs\alpha_S$, gpH_2R-hE2- $Gs\alpha_S$).[26-28] As summarized in Table 5.4, all investigated title compounds exhibited similar potencies and efficacies at mutant hH_2R-gpE2-$Gs\alpha_S$ and gpH_2R-hE2-$Gs\alpha_S$ compared to the corresponding wild-type hH_2R-$Gs\alpha_S$ and gpH_2R-$Gs\alpha_S$, respectively. Hence, these results do not indicate direct interactions of the mutated residues with the bivalent ligands. Furthermore, investigations of selected bivalent acylguanidines on human H_2R mutants (Cys-17→Tyr-17, Ala-271→Asp-271) confirmed that the sensitivity of the double mutant against agonist stimulation is shifted to that of the gpH_2R isoform. Thus, both Tyr-17 in TM1 and Asp-271 in TM7 or at least Asp-271 are key residues for highly potent and efficacious H_2R activation.

Table 5.4. Potencies and efficacies of bivalent acylguanidine-type H_2R agonists at hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$, hH_2R-C17Y-A271D-$Gs\alpha_S$ and hH_2R-C17Y-$Gs\alpha_S$ expressed in Sf9 cell membranes.[a]

Compd.	hH_2R-gpE2-$Gs\alpha_S$		gpH_2R-hE2-$Gs\alpha_S$		hH_2R-C17Y-A271D-$Gs\alpha_S$		hH_2R-C17Y-$Gs\alpha_S$	
	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM	E_{max} ± SEM	pEC_{50} ± SEM
HIS[27-28]	1.00	6.16	1.00	5.85	1.00	6.50	1.00	6.59
AMT[27-28]	0.94 ± 0.05	6.86 ± 0.06	0.94 ± 0.06	6.53 ± 0.09	0.97 ± 0.01	7.19 ± 0.02	0.86 ± 0.19	6.93 ± 0.04
5.26	0.70 ± 0.05	8.05 ± 0.04	0.91 ± 0.03	8.35 ± 0.21	0.79 ± 0.02	8.71 ± 0.12	0.59 ± 0.02	7.87 ± 0.05
5.27	0.77 ± 0.05	8.14 ± 0.03	0.98 ± 0.06	8.65 ± 0.07	0.82 ± 0.03	8.71 ± 0.02	0.65 ± 0.08	8.31 ± 0.31
5.29	0.56 ± 0.03	7.03 ± 0.11	0.96 ± 0.07	7.45 ± 0.01	0.67 ± 0.04	7.39 ± 0.04	0.23 ± 0.02	7.08 ± 0.06
5.47	0.14 ± 0.05	6.71 ± 0.12	0.58 ± 0.05	6.67 ± 0.07	nd[b]		0.13 ± 0.02	6.38 ± 0.06

[a] Steady state GTPase activity in Sf9 membranes expressing hH_2R-gpE2-$Gs\alpha_S$, gpH_2R-hE2-$Gs\alpha_S$, hH_2R-C17Y-A271D-$Gs\alpha_S$ and hH_2R-C17Y-$Gs\alpha_S$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 10 μM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 0.5 and 1.5

pmol·mg^{-1}·min^{-1} for hH_2R-gpE2-Gsα_S and gpH_2R-hE2-Gsα_S, ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-A271D-Gsα_S and ≈ 0.70 and 1.25 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-Gsα_S, and activities stimulated by histamine (100 μM) ranged between ≈ 2.8 and 5.0 pmol·mg^{-1}·min^{-1} for hH_2R-gpE2-Gsα_S and gpH_2R-hE2-Gsα_S, ≈ 1.1 and 4.5 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-A271D-Gsα_S and ≈ 1.1 and 1.8 pmol·mg^{-1}·min^{-1} for hH_2R-C17Y-Gsα_S. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are means ± SEM of one to two experiments performed in duplicate. [b] nd: not determined.

5.3.3 Receptor selectivity

To verify the histamine receptor selectivity profile (human H_2R *vs.* H_1R, H_3R, H_4R) representative compounds were investigated in GTPase assays on recombinant human H_1, H_3 and H_4 receptors for agonism and antagonism, respectively (Table 5.5). In accordance with the results of chapters 3 and 4, all investigated N^G-acylated aminothiazolylpropyl-guanidine-type H_2R agonists proved to be devoid of agonistic and antagonistic activities or to have only negligible effects on histamine receptors other than the H_2R. By contrast, compound **5.27**, which comprises one imidazolylpropylguanidine moiety, showed also significant activities at the other histamine receptor subtypes. In particular, **5.27** turned out to be a highly potent hH_3R and hH_4R partial agonist with EC_{50} values in the low nanomolar range.

Table 5.5. Histamine receptor subtype selectivity of selected bivalent ligands. Agonistic, antagonistic and inverse agonistic effects at hH_1R + RGS4, hH_2R-GsαS, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ + RGS4 and hH_4R-GAIP + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ expressed in Sf9 cell membranes.[a]

Compd.	hH_1R	hH_2R		hH_3R		hH_4R	
	(pK_B)	pEC_{50} (pK_B)	E_{max}	pEC_{50} (pK_B)	E_{max}	pEC_{50} (pK_B)	E_{max}
5.26	(< 6.00)	7.86 ± 0.11	0.75 ± 0.04	(< 5.00)	-	(< 5.00)	-
5.27	(6.27 ± 0.19)	8.12 ± 0.04	0.76 ± 0.05	8.54 ± 0.02	0.68 ± 0.06	8.07 ± 0.09	0.52 ± 0.03
5.29	(< 6.00)	7.16 ± 0.20	0.44 ± 0.05	(< 6.00)	-	(< 6.00)	-
5.31	(6.11 ± 0.03)	6.81 ± 0.11	0.45 ± 0.11	< 6.00	– 0.87 ± 0.12	< 6.00	– 0.66
5.33	(6.18 ± 0.01)	7.66 ± 0.06	0.46 ± 0.03	(< 6.00)	-	(< 6.00)	-
5.39	(< 6.00)	7.50 ± 0.04	0.53 ± 0.02	< 6.00	– 0.50 ± 0.05	< 6.00	– 0.49 ± 0.07

Table 5.5. (continued)

5.41	(6.07 ± 0.03)	7.68 ± 0.11	0.58 ± 0.03	(< 6.00)	-	(< 6.00)	-
5.42	(< 6.00)	7.25 ± 0.21	0.82 ± 0.07	(< 6.00)	-	(< 6.00)	-
5.45	(< 6.00)	6.62 ± 0.23	0.79 ± 0.07	< 5.00	– 0.25	< 5.00	– 0.65
5.46	(< 6.00)	6.82 ± 0.01	0.69 ± 0.11	(< 5.00)	-	(< 5.00)	-
5.49	(< 6.00)	7.13 ± 0.22	0.73 ± 0.06	(< 5.00)	-	(< 6.00)	-
5.63	(< 6.00)	7.32 ± 0.06	0.60 ± 0.03	< 6.00	– 0.41	< 6.00	– 0.39
5.66	(< 6.00)	(7.03 ± 0.10)	-	< 6.00	– 0.31	(< 6.00)	-

[a] Steady state GTPase activity in Sf9 membranes expressing hH_1R+RGS4, hH_2R-GsαS, hH_3R+$G\alpha_{i2}$+$G\beta_1\gamma_2$+RGS4 and hH_4R-GAIP+$G\alpha_{i2}$+$G\beta_1\gamma_2$ was determined as described in *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration-response curves. For antagonism, reaction mixtures contained histamine (hH_1R: 1 µM; hH_3R, hH_4R: 100 nM) and ligands at concentrations from 1 nM to 1 mM. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ≈ 1.5 and 2.5 $pmol \cdot mg^{-1} \cdot min^{-1}$ and activities stimulated by histamine (10 µM) ranged between ≈ 3.5 and 4.5 $pmol \cdot mg^{-1} \cdot min^{-1}$. Data shown are mean values of one to four experiments performed in duplicate. Efficacy (E_{max}) relative to the maximal response of histamine = 1.00. Negative values refer to inverse agonistic effects. IC_{50} values were converted to K_B values using Cheng-Prusoff equation.[38]

5.4 Summary

After successful application of the bivalent ligand approach to acylguanidine-type ligands, the present study was focused on the chemical nature of the spacer as well as on unsymmetrical bivalent ligands bearing two different sets of pharmacophoric groups. The novel H_2R agonists are promising pharmacological tools for more detailed investigations of the H_2R. In agreement with the results of chapter 4, the combination of two hetarylpropylguanidine moieties with decanedioyl spacer resulted in the most potent H_2R agonists. Replacing the second hetarylpropylguanidine moiety with simple alkyl guanidine groups afforded high H_2R agonistic activities (EC_{50} values in the low nanomolar range), whereas all other variations in this part of the molecule led to drastically decreased potencies. A further decrease in potency resulted from the

elimination of the second guanidino group, corroborating the importance of a basic centre at an appropriate distance to the pharmacophore to obtain highly potent bivalent H_2R agonists. These results are consistent with the concept of interaction with the orthosteric and an accessory binding site of one H_2R protomer, i. e. the accessory binding site can accommodate the second acylguanidine portion. Thus, depending on the substitution pattern, the acylguanidine moiety seems to be a versatile structural motif to address an accessory recognition site at the H_2R. To explore the topology of this putative site in more detail, further investigations on H_2R mutants are necessary. Moreover, structural modifications of the spacer (insertion of disulfide, amide, ether as well as rigid phenylene groups) led to drastically decreased potencies, and the combination of an agonistic 3-(2-amino-4-methylthiazol-5-yl)propylguanidine moiety with antagonistic piperidino-methylphenoxypropylamines proved to be inappropriate with respect to H_2R agonistic activities. Actually, the agonistic/antagonistic hybrid molecules showed different qualities of action at hH_2R compared to gpH_2R. It can be speculated if these results indicate a different binding mode for the gpH_2R compared to the hH_2R. In summary, this study substantiates the results obtained with H_2R agonistic twin compounds, suggesting that the increase in potency is due to interaction with an accessory binding site at the same receptor protomer rather than to simultaneous interaction with the orthosteric binding pockets of a hypothetical receptor dimer.

5.5 Experimental section

5.5.1 Chemistry

5.5.1.1 General conditions

See section 3.5.1.1.

The optical rotation (α) was measured on a Perkin-Elmer Polarimeter 241 (Waltham, USA). H_2O/MeCN (1:1) was used as solvent and the polarimeter was thermostated at 20 °C. $[\alpha]_\lambda = \alpha/c \cdot l$; in this equation l is the path length in decimeters and c is the concentration in g/ml for a sample at 20°C and the wavelength λ = 589 nm. The sign of rotation (+ or –) is always given.

5.5.1.2 Preparation of the Boc-protected building block 5.2

10-Benzyloxy-10-oxodecanoic acid (5.1)[43]

Phenylmethanol (0.25 ml, 2.5 mmol) was added dropwise to a cooled suspension of decanedioic acid (0.5 g, 2.5 mmol) and DMAP (cat.) in 3 ml THF/abs. A solution of DCC (0.61 g, 3.0 mmol) in 3 ml THF/abs was added dropwise to this mixture and stirred for 72 hours at ambient temperature. Subsequently, 1,1-dicyclohexylurea was filtered off and the solvent removed under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) to obtain **5.1** (0.34 g, 47 %) as colorless semisolid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 10.88 (s, 1H, COO***H***), 7.34 (m, 5H, Ar-***H***), 5.11 (s, 2H, C***H***$_2$-Ar), 2.34 (m, 4H, COC***H***$_2$), 1.61 (m, 4H, $COCH_2C$***H***$_2$), 1.29 (s, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 179.80 (quat. ***C***OOH), 173.72 (quat. ***C***=O), 136.12 (quat. Ar-***C***), 128.55 (+, Ar-***C***H), 128.18 (+, Ar-***C***H), 66.11 (-, ***C***H_2-Ar), 34.30 (-, ***C***H_2COOH), 34.04 (-, CO***C***H_2), 29.02 (-, ***C***H_2), 28.96 (-, ***C***H_2), 24.90 (-, $COCH_2$***C***H_2), 24.64 (-, ***C***H_2CH_2COOH); EI-MS (70 eV) *m/z* (%): 292 ($M^{+\bullet}$, 30); $C_{17}H_{24}O_4$ (292.37).

10-((*tert*-Butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}aminomethylene)-10-oxodecanoic acid (5.2)

DIEA (0.09 ml, 0.5 mmol) was added to a solution of **5.1** (150 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol) and HOBt-monohydrate (80 mg, 0.5 mmol) in 3 ml DCM/abs under argon and stirred for 15 min. A solution of **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs was added and the mixture stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed *in vacuo*. The crude benzyl-protected compound was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) yielding a pale yellow oil, which was immediately dissolved in 10 ml MeOH and hydrogenated over Pd/C catalyst for 1 h at room temperature. After filtration over Celite, the solvent was removed under reduced pressure to obtain **5.2** (210 mg, 70 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.47 (m, 2H, C***H***$_2$NH), 2.70 (t, ^{3}J = 7.1 Hz, 2H, Thiaz-5-C***H***$_2$), 2.33 (m, 4H, C***H***$_2$COOH, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.64 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2CH_2$COOH), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.33 (s, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 598 (MH^+, 100); $C_{28}H_{47}N_5O_7S$ (597.77).

5.5.1.3 Preparation of the Boc- and Cbz-protected guanidine building blocks 5.3-5.13[44-45]

General procedure

NEt_3 (3 eq) was added to a suspension of the pertinent commercially available amine (1 eq), **3.3** (1 eq) and $HgCl_2$ (2 eq) in DCM/abs and stirred at ambient temperature for 48 h. Subsequently, EtOAc was added and the precipitate filtered over Celite. The crude products were purified by flash chromatography (PE/EtOAc 80/20 v/v) unless otherwise indicated to give the Boc- and Cbz-protected guanidines **5.3-5.13**.

***tert*-Butyl (benzyloxycarbonylamino)(3-phenylpropylamino)methylenecarbamate (5.3)**

The title compound was prepared from 3-phenylpropylamine (0.27 g, 0.28 ml, 2.0 mmol), **3.3** (0.65 g, 2.0 mmol), $HgCl_2$ (1.09 g, 4.0 mmol) and NEt_3 (0.61 g, 0.84 ml, 6.0 mmol) in 10 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.3** (0.69 g, 84 %) as colorless oil. CI-MS (NH_3) m/z (%): 412.3 (MH^+, 100); $C_{23}H_{29}N_3O_4$ (411.5).

***tert*-Butyl (benzyloxycarbonylamino)(3,3-diphenylpropylamino)methylene-carbamate (5.4)**

The title compound was prepared from 3,3-diphenylpropylamine (0.21 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.4** (0.43 g, 88 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.41-7.08 (m, 15H, Ar-***H***), 5.07 (s, 2H, C***H***$_2$-Ar), 3.98 (t, ^3J = 7.68 Hz, 1H, $(Ar)_2C\boldsymbol{H}CH_2$), 3.37 (t, ^3J = 7.14 Hz, 2H, C***H***$_2$NH), 2.33 (m, 2H, CHC***H***$_2$), 1.51 (s, 9H, $(C\boldsymbol{H}_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 488.1 (MH^+, 100); $C_{29}H_{33}N_3O_4$ (487.59).

***tert*-Butyl (benzyloxycarbonylamino)(benzylamino)methylenecarbamate (5.5)**

The title compound was prepared from benzylamine (0.11 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.5** (0.40 g, 100 %) as white foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.45-7.28 (m, 10H, Ar-***H***), 5.16 (s, 2H, C***H***$_2$-Ar), 4.63 (d, ^3J = 5.65 Hz, 2H, C***H***$_2$NH), 1.47 (s, 9H, $(C\boldsymbol{H}_3)_3$); CI-MS (NH_3) m/z (%): 384.2 (MH^+, 100); $C_{21}H_{25}N_3O_4$ (383.44).

(*S*)-*tert*-Butyl (benzyloxycarbonylamino)(1-phenylethylamino)methylenecarbamate (5.6)

The title compound was prepared from (*S*)-1-phenylethylamine (0.18 g, 0.19 ml, 1.5 mmol), **3.3** (0.49 g, 1.5 mmol), $HgCl_2$ (0.81 g, 3.0 mmol) and NEt_3 (0.46 g, 0.65 ml, 4.5 mmol) in 10 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.6** (0.59 g, 99 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.41-7.29 (m, 10H, Ar-***H***), 5.4 (q, ^3J = 6.9 Hz, 1H, Ar-C***H***), 5.13 (s, 2H, Ar-C$\boldsymbol{H}_2$), 1.53 (d, ^3J = 6.9 Hz, 3H, CHC$\boldsymbol{H}_3$)**,** 1.48 (s, 9H, (C$\boldsymbol{H}_3)_3$); CI-MS (NH_3) m/z (%): 398.2 (MH^+, 100); $C_{22}H_{27}N_3O_4$ (397.20).

(*R*)-*tert*-Butyl (benzyloxycarbonylamino)(1-phenylethylamino)methylenecarbamate (5.7)

The title compound was prepared from (*R*)-1-phenylethylamine (0.18 g, 0.19 ml, 1.5 mmol), **3.3** (0.49 g, 1.5 mmol), $HgCl_2$ (0.81 g, 3.0 mmol) and NEt_3 (0.46 g, 0.65 ml, 4.5 mmol) in 10 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.7** (0.63 g, 100 %) as colorless oil. CI-MS (NH_3) m/z (%): 398.3 (MH^+, 100); $C_{22}H_{27}N_3O_4$ (397.20).

***tert*-Butyl (benzyloxycarbonylamino)(4-methoxybenzylamino)methylenecarbamate (5.8)**

The title compound was prepared from 4-methoxybenzylamine (0.20 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure. Purification by flash chromatography (gradient: 0-4 min: PE/EtOAc 100/0, 8-16 min: 90/10, 20-23 min: 60/40) yielded **5.8** (0.28 g, 67 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.40-7.28 (m, 5H, Ar-***H***), 7.24 (d, ^3J = 8.5 Hz, 2H, Ar-***H***), 6.89 (d, ^3J = 8.5 Hz, 2H, Ar-***H***), 5.12 (s, 2H, C$\boldsymbol{H}_2$-Ar), 4.49 (s, 2H, C$\boldsymbol{H}_2$NH), 3.77 (s, 3H, OC$\boldsymbol{H}_3$), 1.50 (s, 9H, (C$\boldsymbol{H}_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 414.1 (MH^+, 100); $C_{22}H_{27}N_3O_5$ (413.47).

***tert*-Butyl (benzyloxycarbonylamino)(3,4-dimethoxybenzylamino)methylene-carbamate (5.9)**

The title compound was prepared from 3,4-dimethoxybenzylamine (0.17 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.9** (0.38 g, 86 %) as yellow oil. CI-MS (NH_3) m/z (%): 443.3 (MH^+, 100); $C_{23}H_{29}N_3O_6$ (443.49).

***tert*-Butyl (benzyloxycarbonylamino)(methylamino)methylenecarbamate (5.10)**

The title compound was prepared from methylamine (0.5 ml 2M in THF, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.10** (0.21 g, 68 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.33 (m, 5H, Ar-***H***), 5.11 (s, 2H, C***H***$_2$-Ar), 2.90 (s, 3H, NHC***H***$_3$), 1.51 (s, 9H, (C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 308 (MH^+, 100); $C_{15}H_{21}N_3O_4$ (307.35).

***tert*-Butyl (benzyloxycarbonylamino)(propylamino)methylenecarbamate (5.11)**

The title compound was prepared from propylamine (0.06 g, 0.08 ml, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.11** (0.28 g, 83 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.32 (m, 5H, Ar-***H***), 5.11 (s, 2H, C***H***$_2$-Ar), 3.33 (m, 2H, C***H***$_2$NH), 1.58 (m, 2H, C***H***$_2$CH$_3$), 1.52 (s, 9H, (C***H***$_3$)$_3$), 0.94 (m, 3H, CH$_2$C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 336 (MH^+, 100); $C_{17}H_{25}N_3O_4$ (335.4).

***tert*-Butyl (benzyloxycarbonylamino)(isobutylamino)methylenecarbamate (5.12)**

The title compound was prepared from isobutylamine (0.07 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.12** (0.30 g, 86 %) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.34 (m, 5H, Ar-***H***), 5.11 (s, 2H, C***H***$_2$-Ar), 2.21 (d, ^3J = 6.9 Hz, 2H, NHC***H***$_2$CH(CH$_3$)$_2$), 1.85 (m, 1H, C***H***(CH$_3$)$_2$), 1.53 (s, 9H, (C***H***$_3$)$_3$), 0.94 (m, 6H, CH(C***H***$_3$)$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 350.1 (MH^+, 100); $C_{18}H_{27}N_3O_4$ (349.42).

***tert*-Butyl (benzyloxycarbonylamino)(*tert*-butyl-3-aminopropylcarbamate)methylenecarbamate (5.13)**

The title compound was prepared from *tert*-butyl 3-aminopropylcarbamate (0.17 g, 1.0 mmol), **3.3** (0.32 g, 1.0 mmol), $HgCl_2$ (0.54 g, 2.0 mmol) and NEt_3 (0.30 g, 0.41 ml, 3.0 mmol) in 8 ml DCM/abs and 10 ml EtOAc according to the general procedure yielding **5.13** (0.40 g, 89 %) as yellow oil. ^{1}H-NMR (DMSO-d$_6$) δ (ppm): 8.48 (m, 1H, N***H***), 7.42-7.29 (m, 5H, Ar-***H***), 6.84 (m, 1H, N***H***), 5.03 (s, 2H, C***H***$_2$-Ar), 3.30 (m, 2H, C***H***$_2$NH), 2.92 (m, 2H, C***H***$_2$NHBoc), 1.58 (m, 2H, C***H***$_2$CH$_2$NH), 1.48 (s, 9H, (C***H***$_3$)$_3$), 1.36 (s, 9H,

($C\boldsymbol{H}_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 451.1 (MH^+, 100); $C_{22}H_{34}N_4O_6$ (450.53).

5.5.1.4 Preparation of the N^G-Boc-protected guanidine building blocks 5.14–5.25[41,46]

General procedure for the synthesis of Boc-protected guanidine building blocks 5.14-5.24

The title compounds were prepared from the corresponding Boc- and Cbz-protected guanidines **5.3-5.13** by hydrogenation over Pd/C (10 %) in a mixture of THF/MeOH (1:1) for 3-5 days at 8 bar (TLC control). After filtration over Celite and washing with MeOH, the solvent was removed *in vacuo* to give the pertinent N^G-Boc-protected guanidine building blocks **5.14-5.24**.

***tert*-Butyl amino(3-phenylpropylamino)methylenecarbamate (5.14)**[47]

The title compound was prepared from **5.3** (0.69 g, 1.7 mmol) and 0.5 g of Pd/C (10 %) in a mixture of 120 ml THF/MeOH (1:1) according to the general procedure yielding **5.14** (0.47 g, 100 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.32-7.14 (m, 5H, Ar-***H***), 3.29 (m, 2H, $C\boldsymbol{H}_2NH$), 2.70 (m, 2H, $C\boldsymbol{H}_2$-Ar), 1.95 (m, 2H, $ArCH_2C\boldsymbol{H}_2$), 1.54 (s, 9H, ($C\boldsymbol{H}_3)_3$); CI-MS (NH_3) m/z (%): 278.2 (MH^+, 100); $C_{15}H_{23}N_3O_2$ (277.36).

***tert*-Butyl amino(3,3-diphenylpropylamino)methylenecarbamate (5.15)**

The title compound was prepared from **5.4** (0.43 g, 0.9 mmol) and 0.45 g of Pd/C (10 %) in a mixture of 140 ml THF/MeOH (1:1) according to the general procedure yielding **5.15** (0.28 g, 88 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.31-7.11 (m, 10H, Ar-***H***), 4.03 (t, ^3J = 7.7 Hz, 1H, $(Ar)_2C\boldsymbol{H}CH_2$), 3.10 (t, ^3J = 7.41 Hz, 2H, $C\boldsymbol{H}_2NH$), 2.31 (m, 2H, $CHC\boldsymbol{H}_2$), 1.44 (s, 9H, ($C\boldsymbol{H}_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 354 (MH^+, 100); $C_{21}H_{27}N_3O_2$ (353.46).

***tert*-Butyl amino(benzylamino)methylenecarbamate (5.16)**[47]

The title compound was prepared from **5.5** (0.4 g, 1.0 mmol) and 0.4 g of Pd/C (10 %) in a mixture of 100 ml THF/MeOH (1:1) according to the general procedure yielding **5.16** (0.21 g, 84 %) as white solid. mp = 127-129 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.4-7.31 (m, 5H, Ar-***H***), 4.51 (m, 2H, $C\boldsymbol{H}_2NH$), 1.47 (s, 9H, ($C\boldsymbol{H}_3)_3$); CI-MS (NH_3) m/z (%): 250.1 (MH^+, 100); $C_{13}H_{19}N_3O_2$ (249.31).

(*S*)-*tert*-Butyl amino(1-phenylethylamino)methylenecarbamate (5.17)

The title compound was prepared from **5.6** (0.59 g, 1.5 mmol) and 0.6 g of Pd/C (10 %) in a mixture of 120 ml THF/MeOH (1:1) according to the general procedure yielding **5.17** (0.36 g, 92 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35 (m, 5H, Ar-***H***), 5.04 (q, ^3J = 7.41 Hz, 1H, Ar-C***H***), 1.59 (d, ^3J = 7.41 Hz, 3H, CHC***H***$_3$)**,** 1.47 (s, 9H, (C***H***$_3$)$_3$); CI-MS (NH_3) m/z (%): 264.2 (MH^+, 100); $C_{14}H_{21}N_3O_2$ (263.34).

(*R*)-*tert*-Butyl amino(1-phenylethylamino)methylenecarbamate (5.18)[47]

The title compound was prepared from **5.7** (0.6 g, 1.5 mmol) and 0.6 g of Pd/C (10 %) in a mixture of 120 ml THF/MeOH (1:1) according to the general procedure yielding **5.18** (0.37 g, 94 %) as colorless solid. mp = 115-117 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35 (m, 5H, Ar-***H***), 5.04 (q, ^3J = 7.41 Hz, 1H, Ar-C***H***), 1.59 (d, ^3J = 7.41 Hz, 3H, CHC***H***$_3$)**,** 1.47 (s, 9H, (C***H***$_3$)$_3$); CI-MS (NH_3) m/z (%): 264.2 (MH^+, 100); $C_{14}H_{21}N_3O_2$ (263.34).

***tert*-Butyl amino(4-methoxybenzylamino)methylenecarbamate (5.19)**[47]

The title compound was prepared from **5.8** (0.27 g, 0.65 mmol) and 0.27 g of Pd/C (10 %) in a mixture of 140 ml THF/MeOH (1:1) according to the general procedure yielding **5.19** (0.15 g, 80 %) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.23 (d, ^3J = 8.51 Hz, 2H, Ar-***H***), 6.90 (d, ^3J = 8.51 Hz, 2H, Ar-***H***), 4.31 (s, 2H, C***H***$_2$NH), 3.77 (s, 3H, OC***H***$_3$), 1.44 (s, 9H, (C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 280.1 (MH^+, 100); $C_{14}H_{21}N_3O_3$ (279.33).

***tert*-Butyl amino(3,4-dimethoxybenzylamino)methylenecarbamate (5.20)**

The title compound was prepared from **5.9** (0.38 g, 0.86 mmol) and 0.4 g of Pd/C (10 %) in a mixture of 80 ml THF/MeOH (1:1) according to the general procedure yielding **5.20** (0.25 g, 94 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.26 (s, 1H, Ar-***H***), 6.99-6.68 (m, 2H, Ar-***H***), 4.53 (s, 2H, Ar-C***H***$_2$), 3.88 (s, 3H, OC***H***$_3$), 3.86 (s, 3H, OC***H***$_3$), 1.48 (s, 9H, (C***H***$_3$)$_3$); CI-MS (NH_3) m/z (%): 310.1 (MH^+, 100); $C_{15}H_{23}N_3O_4$ (309.69).

***tert*-Butyl amino(methylamino)methylenecarbamate (5.21)**[47]

The title compound was prepared from **5.10** (0.21 g, 0.7 mmol) and 0.22 g of Pd/C (10 %) in a mixture of 80 ml THF/MeOH (1:1) according to the general procedure yielding **5.21** (0.12 g, 100 %) as white foam-like solid. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 174 (MH^+, 100), 347.1 ($2MH^+$, 40); $C_7H_{15}N_3O_2$ (173.21).

***tert*-Butyl amino(propylamino)methylenecarbamate (5.22)**[47]

The title compound was prepared from **5.11** (0.27 g, 0.81 mmol) and 0.27 g of Pd/C (10 %) in a mixture of 80 ml THF/MeOH (1:1) according to the general procedure yielding **5.22** (0.16 g, 100 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.12 (t, ^3J = 7.1 Hz, 2H, C***H***$_2$NH), 1.57 (m, 2H, C***H***$_2$CH$_3$), 1.44 (s, 9H, (C***H***$_3$)$_3$), 0.96 (t, ^3J = 7.4 Hz, 3H, CH$_2$C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 202 (MH^+, 100); $C_9H_{19}N_3O_2$ (201.27).

***tert*-Butyl amino(isobutylamino)methylenecarbamate (5.23)**

The title compound was prepared from **5.12** (0.30 g, 0.86 mmol) and 0.30 g of Pd/C (10 %) in a mixture of 140 ml THF/MeOH (1:1) according to the general procedure yielding **5.23** (0.17 g, 92 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 216.2 (MH^+, 100), 257.2 (MH^++MeCN, 80); $C_{10}H_{21}N_3O_2$ (215.29).

***tert*-Butyl amino[(*tert*-butoxycarbonyl)aminopropylamino]methylenecarbamate (5.24)**

The title compound was prepared from **5.13** (0.36 g, 0.8 mmol) and 0.36 g of Pd/C (10 %) in a mixture of 80 ml THF/MeOH (1:1) according to the general procedure yielding **5.24** (0.25 g, 100 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.19 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NHBoc), 3.09 (t, ^3J = 6.6 Hz, 2H, C***H***$_2$NH), 1.68 (m, 2H, C***H***$_2$CH$_2$NH), 1.44 (s, 9H, (C***H***$_3$)$_3$), 1.43 (s, 9H, (C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 317 (MH^+, 100); $C_{14}H_{28}N_4O_4$ (316.4).

***tert*-Butyl amino(3-cyclohexylamino)methylenecarbamate (5.25)**

To a solution of **5.14** (0.14 g, 0.5 mmol) in 30 ml MeOH was added a catalytic amount of Rh/Al_2O_3 and hydrogenated at 7 bar for 4 days. The catalyst was removed by filtration over Celite and washed with MeOH. The solvent was removed under reduced pressure to yield **5.25** (0.09 g, 64 %) without further purification as colorless oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 284.1 (MH^+, 80), 184.1 (MH^+-Boc, 100); $C_{15}H_{29}N_3O_2$ (283.41).

5.5.1.5 Preparation of the piperidinomethylphenoxypropylamine building blocks 5.60 and 5.61

10-Oxo-10-{3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino}decanoic acid (5.60)

DIEA (0.09 ml, 0.5 mmol) was added to a solution of **5.1** (150 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol) and HOBt-monohydrate (80 mg, 0.5 mmol) in 3 ml DCM/abs and stirred for 15 min. A solution of 3-[3-(piperidin-1-ylmethyl)phenoxy]propan-1-amine **5.59**[25] (125 mg, 0.5 mmol) in 2 ml DCM/abs was added and the mixture stirred overnight at room temperature. The solvent was removed under reduced pressure. Thereafter, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed under reduced pressure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-15 min: 75/25, 16-23 min: 50/50, 24-33 min: 20/80) yielded the benzyl-protected **5.60** (120 mg, 46 %) as yellow oil. The intermediate was immediately dissolved in 6 ml MeOH and hydrogenated with Pd/C (60 mg) for 3 h at room temperature. After filtration over Celite, the solvent was evaporated under reduced pressure to obtain **5.60** (80 mg, 37 % overall) as colorless oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.29 (t, ^3J = 7.9 Hz, 1H, Ar-5-***H***), 7.11-6.95 (m, 2H, Ar-2-***H***, Ar-4-***H***), 6.70 (m, 1H, Ar-6-***H***), 4.03 (t, ^3J = 6.1 Hz, 2H, OC***H***$_2$CH$_2$), 3.92 (s, 2H, Pip-N-C***H***$_2$), 3.37 (t, ^3J = 7.2 Hz, 2H, C***H***$_2$NH), 2.86 (m, 4H, Pip-C***H***$_2$), 2.17 (m, 4H, COC***H***$_2$), 1.95 (m, 2H, OCH$_2$C***H***$_2$), 1.74 (m, 4H, Pip-C***H***$_2$), 1.58 (m, 6H, COCH$_2$C***H***$_2$, Pip-4-C***H***$_2$), 1.29 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 433.2 (MH^+, 100); $C_{25}H_{40}N_2O_4$ (432.6).

16-Oxo-16-{3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino}hexadecanoic acid (5.61)

To a solution of hexadecanedioic acid (0.4 g, 1.4 mmol), EDAC (0.27 g, 1.4 mmol) and HOBt-monohydrate (0.22 g, 1.4 mmol) in 3 ml DCM/abs was added DIEA (0.25 ml, 1.4 mmol) and stirred for 10 min. To this mixture a solution of 3-[3-(piperidin-1-ylmethyl)-phenoxy]propan-1-amine **5.59**[25] (0.35 g, 1.4 mmol) in 3 ml DCM/abs was added and stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted three times with EtOAc. After drying over $MgSO_4$, the organic solvent was evaporated. Purification by flash chromatography (PE/EtOAc 70/30-50/50 v/v) yielded **5.61** (0.50 g, 69 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.28 (m, 1H, Ar-5-***H***), 6.98 (m, 3H, Ar-***H***), 4.02 (t, ^3J = 6.9 Hz, 2H, OC***H***$_2$CH$_2$), 3.89 (s, 2H, Pip-N-C***H***$_2$), 3.35 (m, 2H, C***H***$_2$NH), 2.89 (m, 4H, Pip-C***H***$_2$), 2.18 (m, 4H, COC***H***$_2$), 1.97 (m, 2H, OCH$_2$C***H***$_2$), 1.75 (m, 4H, Pip-C***H***$_2$), 1.58 (m, 6H, COCH$_2$C***H***$_2$, Pip-4-C***H***$_2$), 1.28 (m, 20H, (C***H***$_2$)$_{10}$); EI-MS (70 eV) m/z (%): 517.3 (MH^+, 100); $C_{31}H_{52}N_2O_4$ (516.76).

5.5.1.6 Preparation of the N^G-Boc-protected bivalent acylguanidines 5.26a-5.43a and 5.63a-5.66a

General procedure for the synthesis of Boc-protected bivalent acylguanidines 5.26a, 5.27a, and 5.29a-5.42a

DIEA (1 eq) was added to a solution of **5.2** (1 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs and stirred for 15 min. A solution of pertinent guanidine building block **3.18**, **4.8**, **5.14-5.25** or the Boc-protected 2-amino-4-methylthiazol-5-ylpropylamine **3.13** (1 eq) in DCM/abs was added and the pertinent mixture stirred overnight at room temperature. The solvent was removed under reduced pressure. EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) unless otherwise indicated.

***N*^{1}-((*tert*-Butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)-*N*^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)thiazol-5-yl]propylamino}methylene)decanediamide (5.26a)**

The title compound was prepared from **5.2** (135 mg, 0.23 mmol), EDAC (44 mg, 0.23 mmol), HOBt-monohydrate (35 mg, 0.23 mmol), DIEA (0.04 ml, 0.23 mmol) in 3 ml DCM/abs and **3.18** (92 mg, 0.23 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.26a** (120 mg, 57 %) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.05 (s, 1H, Thiaz-4-***H***), 3.47 (m, 4H, C***H***$_2$NH), 2.75 (m, 4H, Thiaz-5-C***H***$_2$), 2.34 (m, 4H, COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.54 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.32 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 979.6 (MH^+, 100); $C_{45}H_{74}N_{10}O_{10}S_2$ (978.50).

***N*^{1}-{(*tert*-Butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}-*N*^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.27a)**

The title compound was prepared from **5.2** (179 mg, 0.3 mmol), EDAC (57 mg, 0.3 mmol), HOBt-monohydrate (46 mg, 0.3 mmol), DIEA (0.05 ml, 0.3 mmol) in 3 ml DCM/abs and **4.8** (120 mg, 0.3 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.27a** (70 mg, 24 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.82 (s,

1H, Im-2-***H***), 7.37-7.22 (m, 16H, Im-5-***H***, CPh_3), 3.38 (m, 4H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 2H, Im-4-C***H***$_2$), 2.71 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 989.7 (MH^+, 100); $C_{54}H_{72}N_{10}O_6S$ (988.54).

N^1-{(*tert*-Butoxycarbonylamino)(3-phenylpropylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.29a)

The title compound was prepared from **5.2** (179 mg, 0.3 mmol), EDAC (57 mg, 0.3 mmol), HOBt-monohydrate (46 mg, 0.3 mmol), DIEA (0.05 ml, 0.3 mmol) in 3 ml DCM/abs and **5.14** (83 mg, 0.3 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.29a** (125 mg, 51 %) as brown oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 857 (MH^+, 100); $C_{43}H_{68}N_8O_8S$ (856.33).

N^1-{(*tert*-Butoxycarbonylamino)(3-phenylbutylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.30a)

The title compound was prepared from **5.2** (100 mg, 0.17 mmol), EDAC (33 mg, 0.17 mmol), HOBt-monohydrate (26 mg, 0.17 mmol), DIEA (0.03 ml, 0.173 mmol) in 4 ml DCM/abs and 1-(3-phenylbutyl)guanidine[21] (33 mg, 0.17 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.30a** (100 mg, 76 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 771.5 (MH^+, 100); $C_{39}H_{62}N_8O_6S$ (771.03).

N^1-{(*tert*-Butoxycarbonylamino)(3,3-diphenylpropylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.31a)

The title compound was prepared from **5.2** (120 mg, 0.2 mmol), EDAC (40 mg, 0.2 mmol), HOBt-monohydrate (32 mg, 0.2 mmol), DIEA (0.04 ml, 0.2 mmol) in 3 ml DCM/abs and **5.15** (78 mg, 0.2 mmol) in 4 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 5-20 min: 80/20, 30-33 min: 50/50) yielded **5.31a** (50 mg, 23 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30-7.15 (m, 10H, Ar-***H***), 3.98 (m, 1H, C***H***$(Ar)_2$), 3.44 (m, 2H, C***H***$_2$NH), 3.38 (m, 2H, C***H***$_2$NH), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.36 (m, 4H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.99-1.79 (m, 4H, Thiaz-5-CH_2C***H***$_2$,

C***H***$_2$CH(Ar)$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 467.3 ((M+2H)$^{2+}$, 100), 933.6 (MH$^+$, 10); $C_{49}H_{72}N_8O_8S$ (933.21).

N^1-{(*tert*-Butoxycarbonylamino)(3-cyclohexylpropylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.32a)

The title compound was prepared from **5.2** (150 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (0.04 ml, 0.25 mmol) in 3 ml DCM/abs and **5.25** (71 mg, 0.25 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.32a** (40 mg, 19 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 863.6 (MH$^+$, 100); $C_{43}H_{74}N_8O_8S$ (863.16).

N^1-{(*tert*-Butoxycarbonylamino)(benzylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.33a)

The title compound was prepared from **5.2** (150 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (0.04 ml, 0.25 mmol) in 3 ml DCM/abs and **5.16** (70 mg, 0.25 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.33a** (90 mg, 45 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 7.32 (m, 5H, Ar-***H***), 4.57 (s, 2H, Ar-C***H***$_2$), 3.39 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 2.73 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.49-2.26 (m, 4H, COC***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.67 (m, 4H, COCH$_2$C***H***$_2$), 1.50 (m, 27H, C(C***H***$_3$)$_3$), 1.36 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 829 (MH$^+$, 100); $C_{41}H_{64}N_8O_8S$ (828.46).

(*S*)-N^1-{(*tert*-Butoxycarbonylamino)(1-phenylethylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.34a)

The title compound was prepared from **5.2** (170 mg, 0.28 mmol), EDAC (54 mg, 0.28 mmol), HOBt-monohydrate (43 mg, 0.28 mmol), DIEA (0.05 ml, 0.28 mmol) in 4 ml DCM/abs and **5.17** (74 mg, 0.28 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.35a** (120 mg, 49 %) as brown oil. EI-MS (70 eV) m/z (%): 843 (MH$^+$, 100); $C_{42}H_{66}N_8O_8S$ (842.32).

(*R*)-N^1-{(*tert*-Butoxycarbonylamino)(1-phenylethylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.35a)

The title compound was prepared from **5.2** (170 mg, 0.28 mmol), EDAC (54 mg, 0.28 mmol), HOBt-monohydrate (43 mg, 0.28 mmol), DIEA (0.05 ml, 0.28 mmol) in 3 ml DCM/abs and **5.18** (74 mg, 0.28 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.34a** (110 mg, 47 %) as brown oil. EI-MS (70 eV) m/z (%): 843.5 (MH^+, 100); $C_{42}H_{66}N_8O_8S$ (842.32).

N^1-{(*tert*-Butoxycarbonylamino)(4-methoxybenzylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.36a)

The title compound was prepared from **5.2** (113 mg, 0.19 mmol), EDAC (37 mg, 0.19 mmol), HOBt-monohydrate (29 mg, 0.19 mmol), DIEA (0.03 ml, 0.19 mmol) in 3 ml DCM/abs and **5.19** (53 mg, 0.19 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 5-20 min: 80/20, 25-30 min: 50/50) yielded **5.36a** (38 mg, 23 %) as colorless oil. EI-MS (70 eV) m/z (%): 430.2 ($(M+2H)^{2+}$, 100), 859.6 (MH^+, 15); $C_{42}H_{66}N_8O_9S$ (859.09).

N^1-{(*tert*-Butoxycarbonylamino)(3,4-dimethoxybenzylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]-propylamino}methylene)decanediamide (5.37a)

The title compound was prepared from **5.2** (150 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (0.04 ml, 0.25 mmol) in 3 ml DCM/abs and **5.20** (77 mg, 0.25 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.37a** (70 mg, 31 %) as yellow oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 889.5 (MH^+, 100); $C_{43}H_{68}N_8O_{10}S$ (889.11).

N^1-{(*tert*-Butoxycarbonylamino)(methylamino)methylene}-N^{10}-((*tert*-butoxy-carbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propyl-amino}methylene)decanediamide (5.38a)

The title compound was prepared from **5.2** (150 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (0.04 ml, 0.25 mmol) in 3 ml DCM/abs and **5.21** (43 mg, 0.25 mmol) in 4 ml DCM/abs according to the general procedure yielding **5.38a** (120 mg, 64 %) as colorless foam-like solid. ^{1}H-NMR ($CDCl_3$)

δ (ppm): 3.47 (m, 2H, C***H***$_2$NH), 2.97 (d, ^3J = 4.7 Hz, 3H, NHC***H***$_3$), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.40 (m, 4H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 377.2 ((M+2H)$^{2+}$, 100), 753.6 (MH$^+$, 25); $C_{35}H_{60}N_8O_8S$ (752.96).

N^1-{(*tert*-Butoxycarbonylamino)(propylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.39a)

The title compound was prepared from **5.2** (160 mg, 0.27 mmol), EDAC (52 mg, 0.27 mmol), HOBt-monohydrate (41 mg, 0.27 mmol), DIEA (0.05 ml, 0.27 mmol) in 6 ml DCM/abs and **5.23** (54 mg, 0.27 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 4-20 min: 80/20) yielded **5.39a** (60 mg, 29 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ(ppm): 3.40 (t, ^3J = 7.1 Hz, 2H, C***H***$_2$NH), 3.32 (m, 2H, C***H***$_2$NH), 2.74 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.42 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.28 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.58 (m, 2H, NHCH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.47 (s, 9H, C(C***H***$_3$)$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$), 0.95 (t, ^3J = 7.86 Hz, 3H, CH$_2$C***H***$_3$); EI-MS (70 eV) m/z (%): 782 (MH$^+$, 100); $C_{37}H_{64}N_8O_8S$ (781.02).

N^1-{(*tert*-Butoxycarbonylamino)(isobutylamino)methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.40a)

The title compound was prepared from **5.2** (150 mg, 0.25 mmol), EDAC (48 mg, 0.25 mmol), HOBt-monohydrate (38 mg, 0.25 mmol), DIEA (0.04 ml, 0.25 mmol) in 3 ml DCM/abs and **5.22** (54 mg, 0.25 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.40a** (120 mg, 60 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.47 (m, 2H, C***H***$_2$NH), 3.26 (m, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.40 (m, 4H, COC***H***$_2$), 2.25 (s, 3H, Thiaz-4-C***H***$_3$), 1.99-1.79 (m, 3H, Thiaz-5-CH$_2$C***H***$_2$, C***H***(CH$_3$)$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.33 (m, 8H, (C***H***$_2$)$_4$), 0.96 (d, ^3J = 6.7 Hz, 6H, CH(C***H***$_3$)$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 398.4 ((M+2H)$^{2+}$, 100), 795.7 (MH$^+$, 30); $C_{38}H_{66}N_8O_8S$ (795.04).

N^1-{(*tert*-Butoxycarbonylamino)[(*tert*-butoxycarbonylaminopropyl)amino]-methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.41a)

The title compound was prepared from **5.2** (210 mg, 0.35 mmol), EDAC (67 mg, 0.35 mmol), HOBt-monohydrate (54 mg, 0.35 mmol), DIEA (0.06 ml, 0.35 mmol) in 3 ml DCM/abs and **5.24** (100 mg, 0.35 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 4-25 min: 80/20) yielded **5.41a** (110 mg, 35 %) as yellow-brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.41 (m, 4H, C***H***$_2$NH), 3.09 (t, ^3J = 6.5 Hz, 2H, C***H***$_2$NHBoc), 2.74 (t, ^3J = 7.3 Hz, 2H, Thiaz-5-C***H***$_2$), 2.42 (t, ^3J = 7.3 Hz, 4H, COC***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 4H, Thiaz-5-CH_2C***H***$_2$, C***H***$_2$$CH_2$NHBoc), 1.68 (m, 4H, $COCH_2$C***H***$_2$), 152-1.43 (m, 36H, (C***H***$_3$)$_3$), 1.37 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 897 (MH^+, 100); $C_{42}H_{73}N_9O_{10}S$ (896.15).

N^1-{3-[2-(*tert*-Butoxycarbonylamino)-4-methylthiazol-5-yl]propyl}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.42a)

The title compound was prepared from **5.2** (66 mg, 0.11 mmol), EDAC (23 mg, 0.11 mmol), HOBt-monohydrate (17 mg, 0.11 mmol), DIEA (0.03 ml, 0.11 mmol) in 3 ml DCM/abs and **3.13** (30 mg, 0.11 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.42a** (15 mg, 16 %) as colorless oil. EI-MS (70 eV) m/z (%): 426.3 ($(M+2H)^{2+}$, 100), 851.6 (MH^+, 15); $C_{40}H_{66}N_8O_8S_2$ (851.13).

N^1-{(*tert*-Butoxycarbonylamino)[3-(1-trityl-1*H*-1,2,4-triazol-5-yl)propylamino]-methylene}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.28a) To a solution of CDI (0.13 g, 0.8 mmol) in 10 ml DMF under argon was added **5.2** (0.4 g, 0.67 mmol) and the mixture was stirred for 1 h. In a second flask, **4.23** (0.28 g, 0.67 mmol) and NaH (60 % dispersion in mineral oil) (0.05 g, 1.34 mmol) in 7 ml DMF under argon was heated to 30-35 °C for 45 min and was then allowed to cool to room temperature. The two mixtures were combined and stirred for 5 h at ambient temperature. Subsequently, water was added and extracted three times with EtOAc. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by flash chromatography

($CHCl_3$/MeOH/NH_3 95/3/2 v/v/v) yielding **5.28a** (0.5 g, 75 %) as brown oil. EI-MS (70 eV) m/z (%): 495.9 ($(M+2H)^{2+}$, 100), 990.5 (MH^+, 20); $C_{53}H_{71}N_{11}O_6S$ (990.27).

General procedure for the synthesis of Boc-protected acylguanidines 5.43a, 5.63a and 5.64a

DIEA (1 eq) was added to a solution of pertinent carboxylic acid (1 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs and stirred for 15 min. A solution of **3.17** (1 eq) in DCM/abs was added and the mixture stirred overnight at room temperature. The solvent was removed under reduced pressure. EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted three times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) unless otherwise indicated.

***N*^{1}-{(*tert*-Butoxycarbonylamino)[3-(1-trityl-1*H*-imidazol-4-yl)propylamino]-methylene}-*N*^{22}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)docosanediamide(5.43a)**

5.43a was prepared from **4.22** (20 mg, 0.04 mmol), EDAC (8 mg, 0.04 mmol), HOBt-monohydrate (6 mg, 0.04 mmol), DIEA (0.07 ml, 0.04 mmol) in 3 ml DCM/abs and **3.17** (16 mg, 0.04 mmol) in 2 ml DCM/abs according to the general procedure without purification yielding **5.43a** (20 mg, 61 %) as sticky yellow oil. EI-MS (70 eV) m/z (%): 408.3 ($(M+2H)^{2+}$, 100), 815.6 (MH^+, 70); $C_{42}H_{74}N_{10}O_4S$ (815.17).

***N*^{1}-{3-[3-(Piperidin-1-ylmethyl)phenoxy]propyl}-*N*^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)-decanediamide (5.63a)**

5.63a was prepared from **5.60** (80 mg, 0.19 mmol), EDAC (36 mg, 0.19 mmol), HOBt-monohydrate (29 mg, 0.19 mmol), DIEA (0.03 ml, 0.19 mmol) in 3 ml DCM/abs and **3.17** (79 mg, 0.19 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 3-12 min: 80/20, 14-22 min: 60/40, 25-40 min: 30/70) yielded **5.63a** (20 mg, 13 %) as pale yellow oil. EI-MS (70 eV) m/z (%): 414.8 ($(M+2H)^{2+}$, 100), 825.6 (MH^+, 15); $C_{43}H_{69}N_7O_7S$ (828.12).

N^1-{3-[3-(Piperidin-1-ylmethyl)phenoxy]propyl}-N^{16}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)hexadecanediamide (5.64a)

5.64a was prepared from **5.61** (100 mg, 0.2 mmol), EDAC (40 mg, 0.2 mmol), HOBt-monohydrate (30 mg, 0.2 mmol), DIEA (0.04 ml, 0.2 mmol) in 4 ml DCM/abs and **3.17** (83 mg, 0.2 mmol) in 2 ml DCM/abs according to the general procedure. Purification by flash chromatography (gradient: 0-2 min: PE/EtOAc 100/0, 5-20 min: 80/20, 25-40 min: 50/50) yielded **5.64a** (40 mg, 22 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.23 (m, 1H, Ar-***H***), 6.91 (m, 3H, Ar-***H***), 4.16 (s, 2H, Pip-N-C***H***$_2$-Ar), 4.10 (m, 2H, OC***H***$_2$CH$_2$), 3.46 (m, 6H, C***H***$_2$NH, Pip-C***H***$_2$), 2.81 (m, 2H, Pip-C***H***$_2$), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.39 (t, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.22 (m, 2H, NHCOC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.03-1.82 (m, 4 H, Thiaz-5-CH$_2$C***H***$_2$, OCH$_2$C***H***$_2$), 1.61 (m, 10H, Pip-C***H***$_2$, Pip-4-C***H***$_2$, COCH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.25 (m, 20H, (C***H***$_2$)$_{10}$); EI-MS (70 eV) m/z (%): 456.9 ((M+2H)$^{2+}$, 100), 912.6 (MH$^+$, 20); $C_{49}H_{81}N_7O_7S$ (912.28).

N^1-{3-(m-Tolyloxy)propyl}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.65a)

5.65a was separated during the purification of **5.63a** by flash chromatography (PE/EtOAc 70/30-50/50 v/v). Yellow oil (20 mg). ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.17 (t, ^3J = 7.7 Hz, 1H, Ar-***H***), 6.92 (m, 3H, Ar-***H***), 4.03 (t, ^3J = 5.8 Hz, 2H, OC***H***$_2$CH$_2$), 3.46 (m, 4H, C***H***$_2$NH), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.38 (t, ^3J = 7.5 Hz, 2H, COC***H***$_2$), 2.33 (s, 3H, Ar-C***H***$_3$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.14 (m, 2H, NHCOC***H***$_2$), 2.04-1.82 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$, OCH$_2$C***H***$_2$), 1.60 (m, 4H, COCH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.31 (m, 8H, (C***H***$_2$)$_4$); EI-MS (70 eV) m/z (%): 373.1 ((M+2H)$^{2+}$, 100), 745.5 (MH$^+$, 30); $C_{38}H_{60}N_6O_7S$ (744.98).

N^1-{6-[3,4-Dioxo-2-(3-(3-(piperidin-1-ylmethyl)phenoxy)propylamino)cyclobut-1-enylamino]hexyl}-N^{10}-((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)decanediamide (5.66a)

CDI (10 mg, 60 µmol) and **5.2** (32 mg, 54 µmol) were dissolved in 2 ml THF/abs and stirred at room temperature until the formation of carbon dioxide ceased. 3-(6-amino-hexylamino)-4-(3-(3-(piperidin-1-ylmethyl)phenoxy)propylamino)cyclobut-3-ene-1,2-

dione **5.62**[25] (16 mg, 36 µmol) dissolved in 2 ml of THF/DMF (1/1) was added, and the solution was stirred overnight at room temperature. The solvent was evaporated and 5 ml water was added. The solution was extracted with $CHCl_3$ and dried over $MgSO_4$. The product was evaporated *in vacuo* yielding **5.66a** (25 mg, 67 %) as yellow oil. EI-MS (70 eV) m/z (%): 511.8 ($(M+2H)^{2+}$, 100), 1022.8 (MH^+, 10); $C_{53}H_{83}N_9O_9S$ (1022.35).

5.5.1.7 Preparation of the N^G-Boc-protected bivalent acylguanidines 5.44a-5.54a

General procedure for the synthesis of Boc-protected bivalent acylguanidines 5.44a-5.51a

To a solution of pertinent dicarboxylic acid (1 eq), EDAC (2 eq) and HOBt-monohydrate (2 eq) in DCM/abs was added DIEA (2 eq) under argon and stirred for 15 min. To this mixture a solution of **3.17** or **3.18** (2 eq) in DCM/abs was added and stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue, the organic phase was separated and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed *in vacuo*. The crude product was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) unless otherwise indicated.

***N*^{1},*N*^{4}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methyl-thiazol-5-yl]propylamino}methylene)benzene-1,4-dicarboxamide (5.44a)**

The title compound was prepared from terephthalic acid (42 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.44a** (120 mg, 51 %) as brown oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.15-7.75 (m, 4H, Ar-***H***), 3.65 (m, 4H, C***H***$_2$NH), 2.76 (m, 4H, Thiaz-5-C***H***$_2$), 2.20 (s, 6H, Thiaz-4-C***H***$_3$), 2.0 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.47 (s, 18H, C(C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 957 (MH^+, 100); $C_{44}H_{64}N_{10}O_{10}S_2$ (957.17).

(1,4-Phenylene)bis(*N*-[(*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene]acetamide) (5.45a)

The title compound was prepared from (1,4-phenylene)diacetic acid (49 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the

general procedure yielding **5.45a** (150 mg, 61 %) as sticky white oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.33-7.10 (m, 4H, Ar-***H***), 3.57 (m, 4H, COC***H***$_2$), 3.32 (m, 4H, C***H***$_2$NH), 2.70 (m, 4H, Thiaz-5-C***H***$_2$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.86 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, (C***H***$_3$)$_3$), 1.49 (s, 9H, (C***H***$_3$)$_3$), 1.47 (s, 9H, (C***H***$_3$)$_3$), 1.44 (s, 9H, (C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 493.4 ((M+2H)$^{2+}$, 100), 985.7 (MH$^+$, 15); $C_{46}H_{68}N_{10}O_{10}S_2$ (985.22).

N^1,N^3-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)cyclopentane-1,3-dicarboxamide (5.46a)

The title compound was prepared from cyclopentane-1,3-dicarboxylic acid (40 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 3 ml DCM/abs according to the general procedure yielding **5.46a** (200 mg, 84 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.49 (m, 4H, C***H***$_2$NH), 2.90-2.55 (m, 6H, Thiaz-5-C***H***$_2$, cPent-***H***), 2.27 (m, 2H, cPent-***H***), 2.21 (s, 6H, Thiaz-4-C***H***$_3$), 2.03-1.84 (m, 8H, cPent-***H***, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.49 (s, 18H, C(C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 475.2 ((M+2H)$^{2+}$, 100), 949.6 (MH$^+$, 10); $C_{43}H_{68}N_{10}O_{10}S_2$ (949.19).

N^4,$N^{4'}$-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)biphenyl-4,4'-dicarboxamide (5.47a)

The title compound was prepared from biphenyl-4,4´-dicarboxylic acid (61 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.47a** (160 mg, 62 %) as white foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.90 (s, 2H, Ar-***H***), 7.87 (s, 2H, Ar-***H***), 7.65 (s, 2H, Ar-***H***), 7.62 (s, 2H, Ar-***H***), 3.67 (m, 4H, C***H***$_2$NH), 2.77 (m, 4H, Thiaz-5-C***H***$_2$), 2.30 (s, 6H, Thiaz-4-C***H***$_3$), 2.0 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.40 (s, 18H, C(C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 1033 (MH$^+$, 100); $C_{50}H_{68}N_{10}O_{10}S_2$ (1033.27).

N^1,$N^{1'}$-(Ethane-1,2-diyl)bis(N^4-(*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)succinamide (5.48a)

The title compound was prepared from 4,4'-[ethane-1,2-diylbis(azanediyl)]bis(4-oxobutanoic acid) (130 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.17** (207 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.48a** (170 mg, 65

%) as brown oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1051.7 (MH^+, 25); $C_{46}H_{74}N_{12}O_{12}S_2$ (1051.28).

N^1,N^{10}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)-5,6-dithiadecanediamide (5.49a)

The title compound was prepared from 5,6-dithiadecanedioicacid (60 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 3 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.49a** (190 mg, 76 %) as yellow oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.07 (s, 2H, Thiaz-4-***H***), 3.51 (m, 4H, C***H***$_2$NH), 2.88-2.65 (m, 8H, Thiaz-5-C***H***$_2$, SC***H***$_2$), 2.57 (m, 4H, COC***H***$_2$), 2.23 (m, 4H, $COCH_2C$***H***$_2$),1.96 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.56 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 501.2 ($(M+2H)^{2+}$, 100), 1001.3 (MH^+, 10); $C_{42}H_{68}N_{10}O_{10}S_4$ (1001.31).

N^1,N^{14}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)-7,8-dithiatetradecanediamide (5.50a)

The title compound was prepared from 7,8-dithiatetradecanedioic acid (74 mg, 0.25 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (77 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 5 ml DCM/abs and **3.18** (200 mg, 0.5 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.50a** (110 mg, 44 %) as brown oil. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 529.3 ($(M+2H)^{2+}$, 100), 1057.5 (MH^+, 25); $C_{46}H_{76}N_{10}O_{10}S_4$ (1057.42).

N^1,N^{26}-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methyl-thiazol-5-yl]propylamino}methylene)-8,19-dioxo-12,15-dioxa-9,18-diazahexacosane-diamide (5.51a)

The title compound was prepared from 8,19-dioxo-12,15-dioxa-9,18-diazahexacosane-1,26-dioic acid[22] (25 mg, 0.05 mmol), EDAC (19 mg, 0.1 mmol), HOBt-monohydrate (15 mg, 0.1 mmol), DIEA (0.02 ml, 0.1 mmol) in 3 ml DCM/abs and **3.17** (41 mg, 0.1 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.51a** (15 mg, 24 %) as sticky white oil. EI-MS (70 eV) m/z (%): 418 ($(M+3H)^{3+}$, 100), 626.5 ($(M+2H)^{2+}$, 30), 1251.5 (MH^+, 5); $C_{58}H_{98}N_{12}O_{14}S_2$ (1251.6).

***N*[1],*N*[11]-Bis((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propylamino}methylene)-3,6,9-trioxaundecanediamide (5.52a)**

The title compound was prepared with minor modification of the general procedure. 3,6,9-Trioxaundecanedioic acid (28 mg, 0.13 mmol, 1 eq), EDAC (60 mg, 0.32 mmol, 2.5 eq), HOBt-monohydrate (49 mg, 0.32 mmol, 2.5 eq) and DIEA (0.09 ml, 0.5 mmol, 4 eq) were dissolved in 3 ml DMF under argon and stirred for 15 min. **3.17** (207 mg, 0.5 mmol) in 2 ml DMF was added and the mixture was allowed to stir overnight at room temperature. After removing of the solvent under reduced pressure, the crude product was dissolved in DCM/abs and extracted with Na_2CO_3 and brine. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to give **5.52a** (80 mg, 60 %) as brown oil, which was used without further purification. ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 507.3 ($(M+2H)^{2+}$, 100), 1013.7 (MH^+, 35); $C_{44}H_{72}N_{10}O_{13}S_2$ (1013.23).

General procedure for the synthesis of Boc-protected bivalent acylguanidines 5.53a and 5.54a

To a solution of pertinent dicarboxylic acid (1 eq), EDAC (2.1 eq) and DMAP (cat.) in DCM/abs/DMF (2/1) was added DIEA (2.1 eq) under argon and stirred for 15 min. To this mixture a solution of **3.17** (2 eq) in DCM/abs was added and stirred overnight at room temperature. The solvent was removed under reduced pressure and MeCN/(10 %) TFA (4/1) was added. Subsequently, the product was purified using preparative RP-HPLC.

***N*[1],*N*[15]-Bis((*tert*-Butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methyl-thiazol-5-yl]propylamino}methylene)-8-[2-(*tert*-butoxycarbonylamino)ethyl]-4,12-dioxo-5,8,11-triazapentadecanediamide (5.53a)**

The title compound was prepared from 8-[2-(*tert*-butoxycarbonylamino)ethyl]-4,12-dioxo-5,8,11-triazapentadecanedioic acid[22] (57 mg, 0.13 mmol), EDAC (52 mg, 0.27 mmol), DMAP (cat.), DIEA (0.05 ml, 0.27 mmol) in 3 ml DCM/abs/DMF (2/1) and **3.17** (105 mg, 0.26 mmol) in 2 ml DCM/abs according to the general procedure yielding **5.53a** (16 mg, 10 %) as colorless foam-like solid. EI-MS (70 eV) m/z (%): 619.2 ($(M+2H)^{2+}$, 100), 1237.6 (MH^+, 10); $C_{55}H_{92}N_{14}O_{14}S_2$ (1237.53).

***N*[1],*N*[15]-Bis((*tert*-Butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methyl-thiazol-5-yl]propylamino}methylene)-9-[2-(*tert*-butoxycarbonylamino)ethyl]-5,13-dioxo-6,9,12-triazapentadecanediamide (5.54a)**

The title compound was prepared from 9-[2-(*tert*-butoxycarbonylamino)ethyl]-5,13-dioxo-6,9,12-triazaheptadecanedioic acid[22] (34 mg, 0.07 mmol), EDAC (29 mg, 0.15 mmol), DMAP (cat.), DIEA (0.03 ml, 0.15 mmol) in 2 ml DCM/abs/DMF (2/1) and **3.17** (60 mg, 0.14 mmol) in 1 ml DCM/abs according to the general procedure yielding **5.54a** (30 mg, 34 %) as colorless foam-like solid. ^{1}H-NMR (CD_3OD) δ (ppm): 3.42 (m, 4H, CONHC***H***$_2$), 3.34 (m, 4H, C***H***$_2$NH), 3.24 (m, 4H, NHC***H***$_2$C***H***$_2$NH$_2$), 3.07 (m, 4H, CONHCH$_2$C***H***$_2$), 2.70 (t, ^3J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.53 (t, ^3J = 6.8 Hz, 4H, COC***H***$_2$), 2.31 (t, ^3J = 7.1 Hz, 4H, NHCOC***H***$_2$), 2.16 (s, 9H, Thiaz-4-C***H***$_3$), 1.92 (m, 8H, Thiaz-5-CH$_2$C***H***$_2$, COCH$_2$C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.49 (s, 18H, C(C***H***$_3$)$_3$), 1.47 (s, 9H, C(C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 633.3 ((M+2H)$^{2+}$, 100), 1265.6 (MH$^+$, 20); $C_{57}H_{96}N_{14}O_{14}S_2$ (1265.59).

5.5.1.8 Preparation of the N^G-Boc-protected trivalent acylguanidine 5.55a

***N*^{1},*N*^{3},*N*^{5}-Tris((*tert*-butoxycarbonylamino){3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propylamino}methylene)benzene-1,3,5-tricarboxamide (5.55a)**

To a solution of benzene-1,3,5-tricarboxylic acid (42 mg, 0.2 mmol), EDAC (125 mg, 0.66 mmol) and HOBt-monohydrate (100 mg, 0.66 mmol) in 3 ml DMF was added DIEA (0.11 ml, 0.66 mmol) and stirred for 15 min. To this mixture a solution of **3.17** (247 mg, 0.6 mmol) in 3 ml DCM/abs was added and stirred overnight at room temperature. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 70/30-60/40 v/v) to give **5.55a** (110 mg, 40 %) as pale yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 8.65 (m, 3H, Ar-***H***), 3.69 (m, 6H, C***H***$_2$NH), 2.80 (m, 6H, Thiaz-5-C***H***$_2$), 2.22 (s, 9H, Thiaz-4-C***H***$_3$), 2.00 (m, 6H, Thiaz-5-CH$_2$C***H***$_2$), 1.51 (s, 27H, C(C***H***$_3$)$_3$), 1.49 (s, 27H, C(C***H***$_3$)$_3$); EI-MS (70 eV) m/z (%): 466.4 ((M+3H)$^{3+}$, 100), 1396.9 (MH$^+$, 20); $C_{63}H_{93}N_{15}O_{15}S_3$ (1396.7).

5.5.1.9 Preparation of the deprotected acylguanidines 5.26-5.55 and 5.63-5.66

General procedure

TFA (20 %) was added to a solution of the protected acylguanidines **5.26a-5.55a** and **5.63a-5.66a** in DCM/abs and the mixture was stirred at ambient temperature until the protecting groups were removed (3-5 h) (TLC control). Subsequently, the solvent was

removed and the residue was purified by preparative RP-HPLC. All compounds were obtained as trifluoroacetic acid salts.

N^1-{[3-(2-Amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-N^{10}-{[3-(2-aminothiazol-5-yl)propylamino](amino)methylene}decanediamide (5.26)

The title compound was prepared from **5.26a** (110 mg, 0.11 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **5.26** as colorless foam-like solid (30 mg, 26 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (m, 4H, C***H***$_2$NH), 2.74 (m, 4H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.41 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.93 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.41 (quat. ***C***=O), 171.82 (quat. Thiaz-2-***C***), 155.29 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.37 (+, Thiaz-4-***C***), 118.44 (quat. Thiaz-5-***C***), 41.47 (-, ***C***H$_2$NH), 37.76 (-, CO***C***H$_2$), 30.20 (-, ***C***H$_2$), 29.97 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.45 (-, COCH$_2$***C***H$_2$), 24.89 (-, Thiaz-5-***C***H$_2$), 11.45 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ([$C_{25}H_{42}N_{10}O_2S_2$ + H]$^+$) calcd. 579.3012, found 579.3006; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65); anal. HPLC: k`= 1.96 (t_R = 9.82 min, column A), purity = 95 %; $C_{25}H_{42}N_{10}O_2S_2$ · 4TFA (1034.88).

N^1-{[3-(1*H*-Imidazol-4-yl)propylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.27)

The title compound was prepared from **5.27a** in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.27** as colorless oil (70 mg, 24 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.82 (s, 1H, Im-2-***H***), 7.37 (s, 1H, Im-5-***H***), 3.38 (m, 4H, C***H***$_2$NH), 2.84 (t, ^3J = 7.7 Hz, 2H, Im-4-C***H***$_2$), 2.71 (t, ^3J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD , 400 MHz, HSQC, HMBC) δ (ppm): 177.37 (quat. ***C***=O), 155.64 (quat. ***C***=NH), 134.96 (quat. Thiaz-***C***-4), 118.46 (quat. Thiaz-***C***-5), 117.09 (+, Im-5-***C***H), 41.56 (-, ***C***H$_2$NH), 37.76 (-, CO***C***H$_2$), 30.13 (-, ***C***H$_2$), 29.96 (-, Thiaz-5-CH$_2$***C***H$_2$), 28.10 (-, Im-4-CH$_2$***C***H$_2$), 25.41 (COCH$_2$***C***H$_2$), 23.60 (-, Thiaz-5-***C***H$_2$), 22.54 (-, Im-4-***C***H$_2$), 11.41 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ([$C_{25}H_{42}N_{10}O_2S$ + H]$^+$) calcd. 547.3291, found 547.3299; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50); anal. HPLC: k`= 1.87 (t_R = 9.51 min, column A), purity = 94 %; $C_{25}H_{42}N_{10}O_2S$ · 4TFA (1002.32).

N^1-{[3-(1*H*-1,2,4-Triazol-5-yl)propylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.28)

The title compound was prepared from **5.28a** (150 mg, 0.15 mmol) in 10 ml DCM/abs and 2 ml TFA according to the general procedure yielding **5.28** as brown oil (20 mg, 13 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.50 (s, 1H, Triaz-5-***H***), 3.40 (m, 4H, C***H***$_2$NH), 2.94 (t, ^3J = 7.4 Hz, 2H, Triaz-3-C***H***$_2$), 2.74 (m, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.41 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.11 (m, 2H, Triaz-3-CH_2C***H***$_2$), 1.93 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, COCH_2C***H***$_2$), 1.36 (m, 8H, (C***H***$_2$)$_4$); HRLSIMS: *m/z* for ([$C_{24}H_{41}N_{11}O_2S$ + H]$^+$) calcd. 548.3244, found 548.3246; prep. HPLC: MeCN/0.1% TFA/aq (10/90-50/50); anal. HPLC: k`= 2.02 (t_R = 8.07 min, column B), purity = 98 %; $C_{24}H_{41}N_{11}O_2S \cdot 4$TFA (1003.8).

N^1-{[3-Phenylpropylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.29)

The title compound was prepared from **5.29a** (130 mg, 0.15 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.29** as colorless oil (40 mg, 48 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.35-7.15 (m, 5H, Ar-***H***), 3.29 (m, 4H, C***H***$_2$NH), 2.71 (m, 4H, Thiaz-5-C***H***$_2$, Ar-C***H***$_2$), 2.45 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.90 (m, 2H, Ar-CH_2C***H***$_2$), 1.67 (m, 4H, COCH_2C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 172.36 (quat. ***C***=O), 129.65 (+, Ar-***C***H), 129.43 (+, Ar-***C***H), 45.61 (-, Ar-***C***H_2), 41.60 (-, ***C***H_2NH), 37.73 (-, CO***C***H_2), 30.36 (-, (***C***H_2)$_2$), 29.95 (-, Thiaz-5-CH_2***C***H_2), 23.64 (-, Thiaz-5-***C***H_2), 11.54 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ([$C_{28}H_{44}N_8O_2S$ + H]$^+$) calcd. 557.3386, found 557.3380; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.26 (t_R = 14.13 min, column A), purity = 96 %; $C_{28}H_{44}N_8O_2S \cdot 3$TFA (898.83).

N^1-{[3-Phenylbutylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.30)

The title compound was prepared from **5.30a** (100 mg, 0.13 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.30** as colorless oil (20 mg, 27 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.25 (m, 5H, Ar-***H***), 3.34 (m, 2H, C***H***$_2$NH), 3.17 (t, ^3J = 6.6 Hz, 2H, C***H***$_2$NH), 2.71 (m, 2H, Thiaz-5-C***H***$_2$), 2.43 (m, 4H, COC***H***$_2$), 2.27 (m, 1H, C***H***CH_3), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.93 (m, 4H, Thiaz-5-CH_2C***H***$_2$, NHCH_2C***H***$_2$), 1.66 (m, 4H, COCH_2C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$), 1.29 (d, ^3J = 6.9 Hz, 3H, CHC***H***$_3$);

HRLSIMS: *m/z* for ($[C_{29}H_{46}N_8O_2S + H]^+$) calcd. 571.3537, found 571.3537; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.02 (t_R = 10.74 min, column B), purity = 96 %; $C_{29}H_{46}N_8O_2S \cdot 3TFA$ (912.85).

N^1-{[3,3-Diphenylpropylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methyl-thiazol-5-yl)propylamino](amino)methylene}decanediamide (5.31)

The title compound was prepared from **5.31a** (44 mg, 0.05 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.31** as brown oil (30 mg, 62 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.27 (m, 8H, Ar-***H***), 7.17 (m, 2H, Ar-***H***), 4.05 (t, ^3J = 7.9 Hz, 1H, C***H***$(Ar)_2$), 3.34 (t, ^3J = 5.7 Hz, 2H, C***H***$_2$NH), 3.24 (t, ^3J = 7.0 Hz, 2H, C***H***$_2$NH), 2.70 (t, ^3J = 7.5 Hz, 2H, Thiaz-5-C***H***$_2$), 2.44 (m, 4H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.97-1.80 (m, 4H, Thiaz-5-CH_2C***H***$_2$, C***H***$_2$CH$(Ar)_2$), 1.64 (m, 4H, $COCH_2$C***H***$_2$), 1.34 (m, 8H, (C***H***$_2)_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.44 (quat. ***C***=O), 177.26 (quat. Thiaz-2-***C***), 155.35 (quat. ***C***=NH), 145.19 (quat. Ar-***C***), 132.65 (quat. Thiaz-4-***C***), 129.77 (+, Ar-***C***H), 128.82 (+, Ar-***C***H), 127.65 (+, Ar-***C***H), 118.41 (quat. Thiaz-5-***C***), 44.04 (+, ***C***H$(Ar)_2$), 41.58 (-, ***C***H_2NH), 41.40 (-, ***C***H_2NH), 37.76 (-, CO***C***H_2), 34.55 (-, ***C***H_2CH$(Ar)_2$), 30.17 (-, (***C***$H_2)_2$), 29.94 (-, (***C***$H_2)_2$), 29.71 (-, Thiaz-5-CH_2***C***H_2), 25.45 (-, $COCH_2$***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 11.45 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{34}H_{48}N_8O_2S + H]^+$) calcd. 633.3699, found 633.3710; prep HPLC: MeCN/0.1 % TFA/aq (25/75-60/40); anal. HPLC: k`= 3.82 (t_R = 12.91 min, column B), purity = 98 %; $C_{34}H_{48}N_8O_2S \cdot 3TFA$ (974.92).

N^1-{[3-Cyclohexylpropylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methyl-thiazol-5-yl)propylamino](amino)methylene}decanediamide (5.32)

The title compound was prepared from **5.32a** (40 mg, 0.05 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.30** as colorless oil (10 mg, 35 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.32 (m, 4H, C***H***$_2$NH), 2.70 (m, 2H, Thiaz-5-C***H***$_2$), 2.43 (m, 4H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.78-1.57 (m, 11H, $COCH_2$C***H***$_2$, cHex-CH_2C***H***$_2$, cHex-***H***), 1.45-1.04 (m, 16H, cHex-C***H***$_2$, cHex-***H***, (C***H***$_2)_4$); HRLSIMS: *m/z* for ($[C_{28}H_{50}N_8O_2S + H]^+$) calcd. 563.3850, found 563.3841; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 5.29 (t_R = 14.15 min, column B), purity = 96 %;

N^1-{[Benzylamino](amino)methylene}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propyl-amino](amino)methylene}decanediamide (5.33)

The title compound was prepared from **5.33a** (90 mg, 0.1 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.33** as colorless oil (40 mg, 76 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.45-7.30 (m, 5H, Ar-***H***), 4.54 (s, 2H, Ar-***C***H_2), 3.29 (m, 2H, C***H***$_2$NH), 2.71 (t, ^{3}J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.43 (quat. ***C***=O), 169.28 (quat. Thiaz-2-***C***), 136.96 (quat. Ar-***C***), 132.92 (quat. Thiaz-4-***C***), 130.13 (+, Ar-***C***H), 129.99 (+, Ar-***C***H), 129.40 (+, Ar-***C***H) 128.42 (+, Ar-***C***H), 118.52 (quat. Thiaz-5-***C***), 45.97 (-, Ar-***C***H_2), 41.60 (-, ***C***H_2NH), 37.85 (-, CO***C***H_2), 30.16 (-, ***C***H_2), 29.95 (-, Thiaz-5-CH_2***C***H_2), 25.37 (-, $COCH_2$***C***H_2), 23.64 (-, Thiaz-5-***C***H_2), 11.47 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{26}H_{40}N_8O_2S + H]^+$) calcd. 529.3073, found 529.3059; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.82 (t_R = 12.67 min, column A), purity = 88 %; $C_{26}H_{40}N_8O_2S \cdot 3TFA$ (870.77).

(*S*)-*N*^{1}-{[1-Phenylethylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.34)

The title compound was prepared from **5.34a** (110 mg, 0.13 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.34** as brown oil (50 mg, 70 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.41-7.3 (m, 5H, Ar-***H***), 3.35 (t, ^{3}J = 7.14 Hz, 2H, C***H***$_2$NH), 3.31 (m, 1H, Ar-C***H***), 2.71 (t, ^{3}J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.59 (d, ^{3}J = 6.9 Hz, 3H, CHC***H***$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.55 (quat. ***C***=O), 166.75 (quat. Thiaz-2-***C***), 141.10 (quat. Ar-***C***-1), 132.87 (quat. Thiaz-4-***C***), 130.19 (+, Ar-***C***H), 129.36 (+, Ar-***C***H), 126.89 (+, Ar-***C***H), 118.46 (quat. Thiaz-5-***C***), 52.92 (+, Ar-***C***H), 37.87 (-, ***C***H_2NH), 37.77 (-, CO***C***H_2), 30.18 (-, ***C***H_2), 29.96 (-, Thiaz-5-CH_2***C***H_2), 25.36 (-, $COCH_2$***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 22.98 (+, ArCHC***H***$_3$), 11.47 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{27}H_{42}N_8O_2S + H]^+$) calcd. 543.3230, found 543.3223; $[\alpha]^{20}_D$ –11.02° ($MeCN/H_2O$ (9:1)); prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.01 (t_R = 13.29 min, column A), purity = 98 %; $C_{27}H_{42}N_8O_2S \cdot 3TFA$ (884.8).

(*R*)-*N*^{1}-{[1-Phenylethylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.35)

The title compound was prepared from **5.35a** (120 mg, 0.14 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.35** as brown oil (60 mg, 79 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.41-7.30 (m, 5H, Ar-***H***), 3.35 (t, ^3J = 7.14 Hz, 2H, C***H***$_2$NH), 3.31 (m, 1H, Ar-C***H***), 2.71 (t, ^3J = 7.7 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.59 (d, ^3J = 6.9 Hz, 3H, CHC***H***$_3$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.55 (quat. ***C***=O), 166.75 (quat. Thiaz-2-***C***), 141.10 (quat. Ar-***C***-1), 132.87 (quat. Thiaz-4-***C***), 130.19 (+, Ar-***C***H), 129.36 (+, Ar-***C***H), 126.89 (+, Ar-***C***H), 118.46 (quat. Thiaz-5-***C***), 52.92 (+, Ar-***C***H), 37.87 (-, ***C***H$_2$NH), 37.77 (-, CO***C***H$_2$), 30.18 (-, ***C***H$_2$), 29.96 (-, Thiaz-5-CH$_2$***C***H$_2$), 25.36 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 22.98 (+, ArCHC***H***$_3$), 11.47 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{27}H_{42}N_8O_2S$ + H]$^+$) calcd. 543.3230, found 543.3224; $[\alpha]^{20}_D$ +12.89° (MeCN/H_2O (9:1)); prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.01 (t_R = 13.31 min, column A), purity = 93 %; $C_{27}H_{42}N_8O_2S \cdot 3TFA$ (884.8).

***N*^{1}-{[4-Methoxylbenzylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.36)**

The title compound was prepared from **5.36a** (36 mg, 0.04 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.36** as a colorless oil (10 mg, 28 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.28 (d, ^3J = 8.7 Hz, 2H, Ar-***H***), 6.94 (m, 2H, Ar-***H***), 4.45 (s, 2H, NHC***H***$_2$Ar), 3.79 (s, 3H, Ar-OC***H***$_3$), 3.35 (t, ^3J = 7.0 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$), 1.34 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.41 (quat. ***C***=O), 161.31 (quat. Ar-***C***), 132.66 (quat. Thiaz-4-***C***), 130.03 (+, Ar-***C***H), 118.44 (quat. Thiaz-5-***C***), 115.47 (+, Ar-***C***H), 55.80 (+, O***C***H$_3$), 45.62 (-, NH***C***H$_2$Ar), 41.61 (-, ***C***H$_2$NH), 37.84 (-, CO***C***H$_2$), 30.19 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.95 (-, (***C***H$_2$)$_4$), 25.36 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 11.47 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{27}H_{42}N_8O_3S$ + H]$^+$) calcd. 559.3179, found 559.3165; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.81 (t_R = 10.19 min, column B), purity = 97 %; $C_{27}H_{42}N_8O_3S \cdot 3TFA$ (900.8).

***N*^{1}-{[3,4-Dimethoxybenzylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methyl-thiazol-5-yl)propylamino](amino)methylene}decanediamide (5.37)**

The title compound was prepared from **5.37a** (70 mg, 0.08 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.37** as a colorless oil (15 mg, 32 %). ^{1}H-NMR (CD_3OD) δ (ppm): 6.95 (m, 3H, Ar-***H***), 4.45 (s, 2H, NHC***H***$_2$Ar), 3.83 (s, 6H, OC***H***$_3$), 3.35 (m, 2H, C***H***$_2$NH), 2.71 (t, ^{3}J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^{3}J = 7.14 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.27 (quat. ***C***=O), 168.22 (quat. Thiaz-2-***C***), 156.63 (quat. ***C***=NH), 132.50 (quat. Thiaz-4-***C***), 118.45 (quat. Thiaz-5-***C***), 41.61 (-, ***C***H$_2$NH), 37.85 (-, CO***C***H$_2$), 30.18 (-, ***C***H$_2$), 29.73 (-, ***C***H$_2$), 29.95 (-, Thiaz-5-CH$_2$-***C***H$_2$), 25,38 (-, CO***C***H$_2$), 23.63 (-, Thiaz-5-***C***H$_2$), 11.47 (+, Thiaz-5-***C***H$_3$); HREIMS: *m/z* for ([$C_{28}H_{44}N_8O_4S$ + H]$^{+}$) calcd. 589.3279, found 589.3274; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.76 (t_R = 10.06 min, column B), purity = 97 %; $C_{28}H_{44}N_8O_4S$ · 3TFA (930.83).

***N*^{1}-{[Methylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)-propylamino](amino)methylene}decanediamide (5.38)

The title compound was prepared from **5.38a** (65 mg, 0.09 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.38** as brown oil (10 mg, 16 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^{3}J = 7.14 Hz, 2H, C***H***$_2$NH), 2.95 (s, 3H, NHC***H***$_3$), 2.71 (t, ^{3}J = 7.41 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (m, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.65 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.40 (quat. ***C***=O), 163.60 (quat. Thiaz-2-***C***), 157.43 (quat. ***C***=NH), 132.65 (quat. Thiaz-4-***C***), 118.45 (quat. Thiaz-5-***C***), 41.61 (-, ***C***H$_2$NH), 37.76 (-, CO***C***H$_2$), 30.19 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.97 (-, (***C***H$_2$)$_4$), 28.32 (+, NH***C***H$_3$), 25.44 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 11.46 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{20}H_{36}N_8O_2S$ + H]$^{+}$) calcd. 453.2760, found 453.2758; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.84 (t_R = 7.59 min, column B), purity = 99 %; $C_{20}H_{36}N_8O_2S$ · 3TFA (794.68).

***N*^{1}-{[Propylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.39)

The title compound was prepared from **5.39a** (50 mg, 0.06 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.39** as yellow oil (30 mg, 60 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^{3}J = 6.9 Hz, 2H, C***H***$_2$NH), 3.25 (t, ^{3}J = 7.3 Hz, 2H, C***H***$_2$NH), 2.71 (t, ^{3}J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^{3}J = 7.4 Hz, 4H, COC***H***$_2$), 2.17

(s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.67 (m, 6H, $COCH_2$C***H***$_2$, C***H***$_2CH_3$), 1.32 (m, 8H, (C***H***$_2$)$_4$), 0.99 (t, ^3J = 7.4 Hz, 3H, CH_2C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.46 (quat. ***C***=O), 170.39 (quat. Thiaz-2-***C***), 155.31 (quat. ***C***=NH), 132.60 (quat. Thiaz-4-***C***), 118.40 (quat. Thiaz-5-***C***), 44.16 (-, ***C***H_2NH), 41.58 (-, ***C***H_2NH), 37.78 (-, CO***C***H_2), 30.15 (-, Thiaz-5-CH_2***C***H_2), 29.93 (-, (***C***H_2)$_4$), 25.43 (-, $COCH_2$***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 22.52 (-, ***C***H_2CH_3), 11.45 (+, Thiaz-4-***C***H_3), 11.33 (+, CH_2***C***H_3); HREIMS: *m/z* for ([$C_{22}H_{40}N_8O_2S$]$^+$) calcd. 480.2995, found 480.2996; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.30 (t_R = 8.83 min, column B), purity = 98 %; $C_{22}H_{40}N_8O_2S$ · 3TFA (822.82).

***N*^{1}-{[Isobutylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)-propylamino](amino)methylene}decanediamide (5.40)**

The title compound was prepared from **5.40a** (100 mg, 0.16 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.40** as brown oil (55 mg, 41 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.39 (t, ^3J = 7.1 Hz, 2H, C***H***$_2$NH), 3.14 (d, ^3J = 7.1 Hz, 2H, NHC***H***$_2$), 2.71 (t, ^3J = 7.4 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.00-1.85 (m, 3H, Thiaz-5-CH_2C***H***$_2$, C***H***$(CH_3)_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$), 1.00 (d, ^3J = 6.9 Hz, 6H, CH(C***H***$_3$)$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.36 (quat. ***C***=O), 156.84 (quat. ***C***=NH), 132.57 (quat. Thiaz-4-***C***), 118.46 (quat. Thiaz-5-***C***), 43.00 (-, ***C***H_2NH), 41.61 (-, ***C***H_2NH), 37.83 (-, CO***C***H_2), 30.22 (-, Thiaz-5-CH_2***C***H_2), 29.98 (-, (***C***H_2)$_4$), 28.83 (+, ***C***$H(CH_3)_2$), 25.44 (-, $COCH_2$***C***H_2), 23.61 (-, Thiaz-5-***C***H_2), 20.07 (+, CH(***C***H_3)$_2$), 11.48 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ([$C_{23}H_{42}N_8O_2S$ + H]$^+$) calcd. 495.3230, found 495.3215; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.61 (t_R = 9.66 min, column B), purity = 99 %; $C_{23}H_{42}N_8O_2S$ · 3TFA (836.76).

***N*^{1}-{[3-Aminopropylamino](amino)methylene}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.41)**

The title compound was prepared from **5.41a** (100 mg, 0.11 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.41** as sticky yellow oil (30 mg, 55 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.42 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 3.35 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 3.02 (m, 2H, C***H***$_2NH_2$), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.3 Hz, 4H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.06-1.84 (m, 4H, Thiaz-5-CH_2C***H***$_2$, C***H***$_2CH_2NH_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.34 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR

(CD_3OD) δ (ppm): 177.43 (quat. ***C***=O), 163.14 (quat. Thiaz-2-***C***), 155.38 (quat. ***C***=NH), 132.60 (quat. Thiaz-4-***C***), 118.40 (quat. Thiaz-5-***C***), 41.57 (-, ***C***H_2NH), 39.50 (-, ***C***H_2NH_2), 38.01 (-, ***C***H_2NH), 37.75 (-, CO***C***H_2), 30.16 (-, Thiaz-5-CH_2***C***H_2), 29.93 (-, (***C***$H_2)_4$), 27.17 (-, ***C***$H_2CH_2NH_2$), 25.43 (-, $COCH_2$***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 11.44 (+, Thiaz-4-***C***H_3); HREIMS: *m/z* for ($[C_{22}H_{41}N_9O_2S + H]^+$) calcd. 496.3182, found 496.3177; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.49 (t_R = 6.66 min, column B), purity = 98 %; $C_{22}H_{41}N_9O_2S \cdot 4TFA$ (951.77).

***N*^{1}-{3-(2-Amino-4-methylthiazol-5-yl)propyl}-*N*^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.42)**

The title compound was prepared from **5.42a** (15 mg, 0.02 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.42** as brown oil (10 mg, 91 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (m, 2H, C***H***$_2$NH), 3.20 (m, 2H, C***H***$_2$NHCO), 2.71 (t, ^3J = 7.68 Hz, 2H, Thiaz-5-C***H***$_2$), 2.62 (t, ^3J = 7.68 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (m, 2H, COC***H***$_2$), 2.18 (m, 2H, COC***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.74 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.69-1.57 (m, 4H, $COCH_2$C***H***$_2$), 1.34 (m, 8H, (C***H***$_2)_4$); HRLSIMS: *m/z* for ($[C_{25}H_{42}N_8O_2S_2 + H]^+$) calcd. 551.2950, found 551.2947; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.90 (t_R = 7.76 min, column B), purity = 96 %; $C_{25}H_{42}N_8O_2S_2 \cdot 3TFA$ (892.84).

***N*^{1}-{[3-(1*H*-Imidazol-4-yl)propylamino](amino)methylene}-*N*^{22}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}docosanediamide (5.43)**

The title compound was prepared from **5.43a** (10 mg, 0.01 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.43** as white foam-like soid (4 mg, 56 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (s, 1H, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.38 (m, 4H, C***H***$_2$NH), 2.83 (m, 2H, Im-4-C***H***$_2$), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^3J = 7.1 Hz, 4H, COC***H***$_2$), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.32 (m, 8H, $COCH_2$(C***H***$_2)_2$), 1.28 (m, 24H, (C***H***$_2)_{12}$); HRLSIMS: *m/z* for ($[C_{37}H_{66}N_{10}O_2S + H]^+$) calcd. 715.5169, found 715.5186; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 4.69 (t_R = 15.23 min, column B), purity = 100 %; $C_{37}H_{66}N_{10}O_2S \cdot 4TFA$ (1171.13).

***N*^{1},*N*^{4}-Bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-benzene-1,4-dicarboxamide (5.44)**

The title compound was prepared from **5.44a** (120 mg, 0.13 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.44** as yellow oil (40 mg, 57 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.16 (m, 4H, Ar-***H***), 3.46 (t, ^3J = 7.1 Hz, 4H, C***H***$_2$NH), 2.76 (t, ^3J = 7.7 Hz, 4H, Thiaz-5-C***H***$_2$), 2.20 (s, 6H, Thiaz-4-C***H***$_3$), 1.97 (m, 4H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.47 (quat. ***C***=O), 132.65 (quat. Thiaz-4-***C***), 131.20 (quat. Ar-***C***), 129.95 (quat. Ar-***C***), 118.43 (quat. Thiaz-5-***C***), 41.98 (-, ***C***H_2NH), 29.78 (-, Thiaz-5-CH_2***C***H_2), 23.64 (-, Thiaz-5-***C***H_2), 11.48 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{24}H_{32}N_{10}O_2S_2 + H]^+$) calcd. 557.2229, found 557.2225; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.48 (t_R = 8.23 min, column A), purity = 85 %; $C_{24}H_{32}N_{10}O_2S_2 \cdot 4$TFA (1012.79).

(1,4-Phenylene)bis(*N*-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)-methylene}acetamide) (5.45)

The title compound was prepared from **5.45a** (100 mg, 0.1 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.45** as white foam-like solid (25 mg, 43 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.31 (s, 4H, Ar-***H***), 3.79 (s, 4H, COC***H***$_2$), 3.34 (t, ^3J = 6.86 Hz, 4H, C***H***$_2$NH), 2.71 (t, ^3J = 7.41 Hz, 4H, Thiaz-5-C***H***$_2$), 2.16 (s, 6H, Thiaz-4-C***H***$_3$), 1.89 (m, 4H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 180.18 (quat. ***C***=O), 175.11 (quat. Thiaz-2-***C***), 155.38 (quat. ***C***=NH), 133.74 (quat. Ar-***C***), 132.63 (quat. Thiaz-4-***C***), 131.01 (+, Ar-***C***H), 118.42 (quat. Thiaz-5-***C***), 43.95 (-, CO***C***H_2), 41.66 (-, ***C***H_2NH), 29.67 (-, Thiaz-5-CH_2***C***H_2), 23.61 (-, Thiaz-5-***C***H_2), 11.46 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{26}H_{36}N_{10}O_2S_2 + H]^+$) calcd. 585.2542, found 585.2558; prep HPLC: MeCN/0.1 % TFA/aq (10/90-40/60); anal. HPLC: k`= 1.28 (t_R = 6.09 min, column B), purity = 94 %; $C_{26}H_{36}N_{10}O_2S_2 \cdot 4$TFA (1040.84).

***N*^{1},*N*^{3}-Bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-cyclopentane-1,3-dicarboxamide (5.46)**

The title compound was prepared from **5.46a** (180 mg, 0.19 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.46** as brown oil (60 mg, 58 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, ^3J = 6.9 Hz, 4H, C***H***$_2$NH), 3.04 (m, 2H, cPent-***H***), 2.72 (t, ^3J = 7.6 Hz, 4H, Thiaz-5-C***H***$_2$), 2.26 (m, 2H, cPent-***H***), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 2.02 (m, 4H, cPent-***H***), 1.91 (m, 4H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 170.37 (quat. ***C***=O), 132.54 (quat. Thiaz-4-***C***), 118.52 (quat. Thiaz-5-***C***), 47.16 (-, CO***C***H_2), 41.68 (-, ***C***H_2NH), 37.00 (-, cPent-2-***C***), 30.53 (-, cPent-***C***), 29.69 (-, Thiaz-5-

$CH_2\boldsymbol{C}H_2$), 23.65 (-, Thiaz-5-$\boldsymbol{C}H_2$), 11.45 (+, Thiaz-4-$\boldsymbol{C}H_3$); HREIMS: *m/z* for ($[C_{23}H_{36}N_{10}O_2S_2 + H]^+$) calcd. 549.2537, found 549.2540; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 1.03 (t_R = 5.43 min, column B), purity = 94 %; $C_{23}H_{36}N_{10}O_2S_2 \cdot 4TFA$ (1004.81).

N^4,$N^{4'}$-Bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-biphenyl-4,4'-dicarboxamide (5.47)

The title compound was prepared from **5.47a** (150 mg, 0.15 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.47** as brown oil (52 mg, 55 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.14 (d, ^3J = 8.5 Hz, 4H, Ar-***H***), 7.93 (d, ^3J = 8.5 Hz, 4H, Ar-***H***) 3.46 (t, ^3J = 7.1 Hz, 4H, C$\boldsymbol{H}_2$NH), 2.77 (t, ^3J = 7.7 Hz, 4H, Thiaz-5-C$\boldsymbol{H}_2$), 2.20 (s, 6H, Thiaz-4-C$\boldsymbol{H}_3$), 1.97 (m, 4H, Thiaz-5-$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.67 (quat. ***C***=O), 132.65 (quat. Thiaz-4-***C***), 130.22 (quat. Ar-***C***), 129.93 (quat. Ar-***C***), 118.40 (quat. Thiaz-5-***C***), 41.95 (-, ***C***H_2NH), 29.95 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 23.70 (-, Thiaz-5-***C***H_2), 11.48 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{30}H_{36}N_{10}O_2S_2 + H]^+$) calcd. 633.2542, found 633.2554; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.98 (t_R = 9.88 min, column A), purity = 90 %; $C_{24}H_{32}N_{10}O_2S_2 \cdot 4TFA$ (1088.88).

N^1,$N^{1'}$-(Ethane-1,2-diyl)bis{N^4-[3-(2-amino-4-methylthiazol-5-yl)propyl-amino](amino)methylene}succinamide (5.48)

The title compound was prepared from **5.48a** (100 mg, 0.1 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.48** as brown oil (25 mg, 38 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.36 (m, 4H, C$\boldsymbol{H}_2$NH), 3.21 (t, ^3J = 7.0 Hz, 4H, CONHC$\boldsymbol{H}_2$), 2.78-2.34 (m, 12H, Thiaz-5-C$\boldsymbol{H}_2$, COC$\boldsymbol{H}_2$), 2.17 (s, 6H, Thiaz-4-C$\boldsymbol{H}_3$), 1.87 (m, 4H, Thiaz-5-$CH_2C\boldsymbol{H}_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 170.34 (quat. ***C***=O), 158.81 (quat. ***C***=NH), 132.48 (quat. Thiaz-4-***C***), 118.51 (quat. Thiaz-5-***C***), 41.60 (-, ***C***H_2NH), 41.36 (-, CO***C***H_2), 30.52 (CONH***C***H_2) 29.11 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 23.45 (-, Thiaz-5-***C***H_2), 11.41 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ($[C_{26}H_{42}N_{12}O_4S_2 + H]^+$) calcd. 651.2966, found 651.2966; prep HPLC: MeCN/0.1 % TFA/aq (10/90-30/70); anal. HPLC: k`= 1.51 (t_R = 6.73 min, column B), purity = 98 %; $C_{26}H_{42}N_{12}O_4S_2 \cdot 4TFA$ (1106.9).

N^1,N^{10}-Bis({3-[2-amino-4-methylthiazol-5-yl]propylamino}(amino)methylene)-5,6-dithiadecanediamide (5.49)

The title compound was prepared from **5.49a** (190 mg, 0.19 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.49** as yellow oil (60 mg, 53 %).

^{1}H-NMR (CD_3OD) δ (ppm): 7.01 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.1 Hz, 4H, C***H***$_2$NH), 2.76 (m, 8H, Thiaz-5-C***H***$_2$, SC***H***$_2$), 2.62 (t, ^3J = 7.41 Hz, 4H, COC***H***$_2$), 2.05 (m, 4H, $COCH_2C$***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.64 (quat. ***C***=O), 171.84 (quat. Thiaz-2-***C***), 155.26 (quat. ***C***=NH), 126.35 (quat. Thiaz-5-***C***), 123.30 (+, Thiaz-4-***C***), 41.49 (-, ***C***H_2NH), 37.98 (-, CO***C***H_2), 35.99 (-, S***C***H_2), 29.49 (-, Thiaz-5-CH_2-***C***H_2), 24.87 (-, Thiaz-5-***C***H_2), 24.52 (-, $COCH_2$***C***H_2); HRLSIMS: *m/z* for ($[C_{22}H_{36}N_{10}O_2S_4 + H]^+$) calcd. 601.1984, found 601.1972; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 1.56 (t_R = 6.85 min, column B), purity = 97 %; $C_{22}H_{36}N_{10}O_2S_4 \cdot 4$TFA (1056.93).

***N*^{1},*N*^{14}-Bis({3-[2-amino-4-methylthiazol-5-yl]propylamino}(amino)methylene)-7,8-dithiatetradecanediamide (5.50)**

The title compound was prepared from **5.50a** (100 mg, 0.1 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.50** as yellow oil (41 mg, 62 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.00 (s, 2H, Thiaz-4-***H***), 3.37 (t, ^3J = 7.0 Hz, 4H, C***H***$_2$NH), 2.76 (t, ^3J = 7.5 Hz, 4H, Thiaz-5-C***H***$_2$), 2.69 (t, ^3J = 7.0 Hz, 4H, SC***H***$_2$), 2.49 (t, ^3J = 7.3 Hz, 4H, COC***H***$_2$), 1.95 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.69 (m, 8H, SCH_2C***H***$_2$, $COCH_2C$***H***$_2$), 1.46 (m, 4H, C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.23 (quat. ***C***=O), 171.80 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.51 (+, Thiaz-4-***C***), 41.49 (-, ***C***H_2NH), 39.24 (-, S***C***H_2), 37.98 (-, CO***C***H_2), 29.77 (-, SCH_2***C***H_2), 29.52 (-, Thiaz-5-CH_2-***C***H_2), 28.68 (-, SCH_2CH_2***C***H_2), 24.99 (-, $COCH_2$***C***H_2), 24.89 (-, Thiaz-5-***C***H_2); HRLSIMS: *m/z* for ($[C_{26}H_{44}N_{10}O_2S_4 + H]^+$) calcd. 657.2604, found 657.2599; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.43 (t_R = 9.18 min, column B), purity = 98 %; $C_{26}H_{44}N_{10}O_2S_4 \cdot 4$TFA (1113.03).

***N*^{1},*N*$^{1'}$-((Ethane-1,2-diyldioxy)bis[ethane-2,1-diyl])bis-*N*^{8}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}octanediamide (5.51)**

The title compound was prepared from **5.51a** (15 mg, 0.01 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.51** as yellow oil (5 mg, 57 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.61 (m, 4H, $NHCH_2C$***H***$_2$), 3.53 (t, ^3J = 5.5 Hz, 4H, OC***H***$_2$), 3.34 (m, 8H, C***H***$_2$NH, CONHC***H***$_2$), 2.71 (m, 4H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.3 Hz, 4H, COC***H***$_2$), 2.21 (m, 4H, NHCOC***H***$_2$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.88 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.63 (m, 8H, $COCH_2C$***H***$_2$), 1.37 (m, 8H, (C***H***$_2$)$_2$); EI-MS (70 eV) m/z (%): 298.1 ($(M+3H)^{3+}$, 100), 426.1 ($(M+2H)^{2+}$, 50), 851.7 (MH^+, 10); HRLSIMS: *m/z* for

($[C_{38}H_{66}N_{12}O_6S_2 + H]^+$) calcd. 851.4742, found 851.4740; prep HPLC: MeCN/0.1 % TFA/aq (10/90-35/65); anal. HPLC: k`= 2.47 (t_R = 9.30 min, column B), purity = 97 %; $C_{38}H_{66}N_{12}O_6S_2 \cdot 4TFA$ (1307.22).

N^1,N^{11}-Bis{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-3,6,9-trioxaundecanediamide (5.52)

The title compound was prepared from **5.52a** (80 mg, 0.08 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.52** as colorless oil (25 mg, 51 %). ^{1}H-NMR (CD_3OD) δ (ppm): 4.25 (s, 2H, COC***H***$_2$), 4.15 (s, 2H, COC***H***$_2$), 3.74 (m, 8H, OC***H***$_2$), 3.38 (t, ^3J = 6.9 Hz, 2H, C***H***$_2$NH), 3.21 (t, ^3J = 7.0 Hz, 2H, C***H***$_2$NH), 2.69 (m, 4H, Thiaz-5-C***H***$_2$), 2.17 (s, 6H, Thiaz-4-C***H***$_3$), 1.85 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 174.02 (quat. ***C***=O), 132.48 (quat. Thiaz-4-***C***), 118.51 (quat. Thiaz-5-***C***), 72.21 (-, COC***H***$_2$), 71.24 (-, OC***H***$_2$), 41.76 (-, ***C***H$_2$NH), 41.36 (-, ***C***H$_2$NH), 30.52 (-, Thiaz-5-CH$_2$-***C***H$_2$), 29.78 (-, Thiaz-5-CH$_2$-***C***H$_2$), 23.56 (-, Thiaz-5-***C***H$_2$), 23.45 (-, Thiaz-5-***C***H$_2$), 11.47 (+, Thiaz-4-***C***H$_3$), 11.41 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ($[C_{24}H_{40}N_{10}O_5S_2 + H]^+$) calcd. 613.2697, found 613.2698; prep HPLC: MeCN/0.1 % TFA/aq (10/90-30/70); anal. HPLC: k`= 0.77 (t_R = 4.73 min, column B), purity = 90 %; $C_{24}H_{40}N_{10}O_5S_2 \cdot 4TFA$ (1068.85).

N^1,N^{15}-Bis({3-[2-amino-4-methylthiazol-5-yl]propylamino}(amino)methylene)-8-[2-aminoethyl]-4,12-dioxo-5,8,11-triazapentadecanediamide (5.53)

The title compound was prepared from **5.53a** (26 mg, 0.02 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.53** as sticky yellow oil (11 mg, 75 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.41 (m, 4H, CONHC***H***$_2$), 3.35 (t, ^3J = 6.8 Hz, 4H, C***H***$_2$NH), 3.20 (m, 4H, NC***H***$_2$C***H***$_2$NH$_2$), 3.02 (m, 4H, CONHCH$_2$C***H***$_2$), 2.78 (m, 4H, Thiaz-5-C***H***$_2$), 2.71 (t, ^3J = 7.6 Hz, 4H, COC***H***$_2$), 2.60 (t, ^3J = 6.2 Hz, 4H, NHCOC***H***$_2$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.51 (quat. ***C***=O), 170.36 (quat. Thiaz-2-***C***), 159.85 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.43 (quat. Thiaz-5-***C***), 55.87 (-, N***C***H$_2$), 41.60 (-, ***C***H$_2$NH), 37.86 (-, ***C***H$_2$NH$_2$), 37.66 (-, CONH***C***H$_2$), 32.76 (-, NHCO***C***H$_2$), 30.21 (-, CO***C***H$_2$), 29.75 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.61 (-, Thiaz-5-***C***H$_2$), 11.46 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ($[C_{30}H_{52}N_{14}O_4S_2 + H]^+$) calcd. 737.3810, found 737.3814; prep HPLC: MeCN/0.1 % TFA/aq (10/90-40/60); anal. HPLC: k`= 0.29 (t_R = 3.46 min, column B), purity = 97 %; $C_{30}H_{52}N_{14}O_4S_2 \cdot 4TFA$ (1193.04).

N^1,N^{17}-Bis({3-[2-amino-4-methylthiazol-5-yl]propylamino}(amino)methylene)-9-[2-aminoethyl]-5,13-dioxo-6,9,12-triazaheptadecanediamide (5.54)

The title compound was prepared from **5.54a** (30 mg, 0.02 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.54** as sticky yellow oil (17 mg, 70 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.41 (m, 4H, CONHC***H***$_2$), 3.34 (m, 4H, C***H***$_2$NH), 3.21 (m, 4H, NC***H***$_2$C***H***$_2$NH$_2$), 3.04 (m, 4H, CONHCH$_2$C***H***$_2$), 2.70 (t, ^3J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.53 (t, ^3J = 6.9 Hz, 4H, COC***H***$_2$), 2.31 (t, ^3J = 7.0 Hz, 4H, NHCOC***H***$_2$), 2.17 (s, 6H, Thiaz-4-C***H***$_3$), 1.91 (m, 8H, Thiaz-5-CH$_2$C***H***$_2$, COCH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.76 (quat. ***C***=O), 176.46 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 132.60 (quat. Thiaz-4-***C***), 118.43 (quat. Thiaz-5-***C***), 55.92 (-, N***C***H$_2$), 41.61 (-, ***C***H$_2$NH), 37.60 (-, ***C***H$_2$NH$_2$), 36.79 (-, CONH***C***H$_2$), 35.50 (-, CO***C***H$_2$), 29.72 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.63 (-, Thiaz-5-***C***H$_2$), 21.18 (-, COCH$_2$***C***H$_2$), 11.46 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ([$C_{32}H_{56}N_{14}O_4S_2$ + H]$^+$) calcd. 765.4129, found 765.4116; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 0.57 (t_R = 4.21 min, column B), purity = 100 %; $C_{32}H_{56}N_{14}O_4S_2$ · 4TFA (1221.09).

N^1,N^3,N^5-Tris{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-benzene-1,3,5-tricarboxamide (5.55)

The title compound was prepared from **5.55a** (100 mg, 0.07 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.55** as colorless oil (35 mg, 60 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.87 (s, 3H, Ar-***H***), 3.46 (t, ^3J = 6.9 Hz, 6H, C***H***$_2$NH), 2.76 (t, ^3J = 7.4 Hz, 6H, Thiaz-5-C***H***$_2$), 2.19 (s, 9H, Thiaz-4-C***H***$_3$), 1.97 (m, 6H, Thiaz-5-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 170.39 (quat. ***C***=O), 168.04 (quat. Thiaz-2-***C***), 155.69 (quat. ***C***=NH), 134.77 (quat. Ar-***C***), 133.93 (+, Ar-***C***H), 132,65 (quat. Thiaz-4-***C***), 118,38 (quat. Thiaz-5-***C***), 41.96 (-, ***C***H$_2$NH), 29.73 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 11.47 (+, Thiaz-4-***C***H$_3$); HRLSIMS: *m/z* for ([$C_{33}H_{45}N_{15}O_3S_3$ + H]$^+$) calcd. 796.3070, found 796.3060; prep HPLC: MeCN/0.1 % TFA/aq (10/90-50/50); anal. HPLC: k`= 1.24 (t_R = 6.00 min, column B), purity = 94 %; $C_{33}H_{45}N_{15}O_3S_3$ · 6TFA (1480.12).

N^1-{3-[3-(Piperidin-1-ylmethyl)phenoxy]propyl}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}decanediamide (5.63)

The title compound was prepared from **5.63a** (20 mg, 0.024 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.63** as colorless oil (15 mg, 93 %).

^{1}H-NMR (CD_3OD) δ (ppm): 7.38 (t, ^{3}J =7.8 Hz, 1H, Ar-***H***), 7.06 (m, 2H, Ar-***H***), 7.03 (m, 1H, Ar-***H***), 4.23 (s, 2H, Pip-N-C***H***$_2$-Ar), 4.04 (t, ^{3}J = 6.1 Hz, 2H, OC***H***$_2$CH$_2$), 3.43 (m, 2H, Pip-C***H***$_2$), 3.35 (t, ^{3}J =6.9 Hz, 4H, C***H***$_2$NH), 2.95 (m, 2H, Pip-C***H***$_2$), 2.71 (t, ^{3}J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^{3}J = 7.4 Hz, 2H, COC***H***$_2$), 2.17 (m, 5H, Thiaz-4-C***H***$_3$, NHCOC***H***$_2$), 1.96 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$, OCH$_2$C***H***$_2$), 1.80 (m, 4H, Pip-C***H***$_2$), 1.57 (m, 6H, COCH$_2$C***H***$_2$, Pip-4-C***H***$_2$), 1.31 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.45 (quat. ***C***=O), 176.46 (quat. ***C***=O), 170.23 (quat. Thiaz-2-***C***), 161.02 (quat. Ar-***C***), 155.37 (quat. ***C***=NH), 132.60 (quat. Thiaz-4-***C***), 131.78 (quat. Ar-***C***), 131.50 (+, Ar-***C***H), 124.36 (+, Ar-***C***H), 118.42 (quat. Thiaz-5-***C***), 118.38 (+, Ar-***C***H), 117.14 (+, Ar-***C***H), 66.78 (-, ***C***H$_2$-OAr), 61.71 (+, Pip-N-***C***H$_2$), 54.05 (+, Pip-2-***C***H$_2$, Pip-6-***C***H$_2$), 41.58 (-, ***C***H$_2$NH), 37.76 (-, CONH***C***H$_2$), 37.38 (-, CO***C***H$_2$), 37.15 (-, CO***C***H$_2$), 30.26 (-, (***C***H$_2$)$_2$), 29.97 (-, (***C***H$_2$)$_2$), 29.72 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.04 (-, ***C***H$_2$CH$_2$O), 25.46 (-, COCH$_2$***C***H$_2$), 24.10 (-, Pip-3-***C***H$_2$, Pip-5-***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 22.76 (-, Pip-4-***C***H$_2$), 11.45 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{33}H_{53}N_7O_3S$]$^{+\bullet}$) calcd. 627.3931, found 627.3933; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.39 (t_R = 9.08 min, column B), purity = 99 %; $C_{33}H_{53}N_7O_3S \cdot 3TFA$ (969.94).

N^{1}-{3-[3-(Piperidin-1-ylmethyl)phenoxy]propyl}-N^{16}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)methylene}hexadecanediamide (5.64)

The title compound was prepared from **5.64a** (40 mg, 0.044 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.64** as white foam-like solid (15 mg, 48 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.38 (t, ^{3}J =7.8 Hz, 1H, Ar-***H***), 7.06 (m, 2H, Ar-***H***), 7.03 (m, 1H, Ar-***H***), 4.23 (s, 2H, Pip-N-C***H***$_2$-Ar), 4.04 (t, ^{3}J = 6.1 Hz, 2H, OC***H***$_2$CH$_2$), 3.43 (m, 2H, Pip-C***H***$_2$), 3.35 (m, 4H, C***H***$_2$NH), 2.94 (t, ^{3}J = 12.5 Hz, 2H, Pip-C***H***$_2$), 2.71 (t, ^{3}J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.46 (t, ^{3}J = 7.4 Hz, 2H, COC***H***$_2$), 2.17 (m, 5H, Thiaz-4-C***H***$_3$, NHCOC***H***$_2$), 2.03-1.69 (m, 10 H, Thiaz-5-CH$_2$C***H***$_2$, OCH$_2$C***H***$_2$, Pip-C***H***$_2$), 1.59 (m, 4H, COCH$_2$C***H***$_2$), 1.28 (m, 20H, (C***H***$_2$)$_{10}$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.45 (quat. ***C***=O), 176.49 (quat. ***C***=O), 170.38 (quat. Thiaz-2-***C***), 161.03 (quat. Ar-***C***), 132.62 (quat. Thiaz-4-***C***), 131.76 (quat. Ar-***C***), 131.51 (+, Ar-***C***H), 124.35 (+, Ar-***C***H), 118.44 (quat. Thiaz-5-***C***), 118.44 (+, Ar-***C***H), 117.12 (+, Ar-***C***H), 66.80 (-, ***C***H$_2$-OAr), 61.71 (+, Pip-N-***C***H$_2$), 54.06 (+, Pip-2-***C***H$_2$, Pip-6-***C***H$_2$), 41.60 (-, ***C***H$_2$NH), 37.79 (-, CONH***C***H$_2$), 37.38 (-, CO***C***H$_2$), 37.19 (-, CO***C***H$_2$), 30.79 (-, ***C***H$_2$), 30.68 (-, ***C***H$_2$), 30.50 (-, ***C***H$_2$), 30.40 (-, ***C***H$_2$), 30.31 (-, ***C***H$_2$), 30.04 (-, ***C***H$_2$), 29.72 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.11 (-, ***C***H$_2$CH$_2$O), 25.51 (-, COCH$_2$***C***H$_2$), 24.11 (-, Pip-3-***C***H$_2$, Pip-5-***C***H$_2$), 23.62 (-, Thiaz-5-

CH_2), 22.75 (-, Pip-4-***C***H_2), 11.46 (+, Thiaz-4-***C***H_3); HRLSIMS: *m/z* for ([$C_{39}H_{65}N_7O_3S$ + H]$^+$) calcd. 712.4948, found 712.4944; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.96 (t_R = 13.27 min, column B), purity = 100 %; $C_{39}H_{65}N_7O_3S$ · 3TFA (1054.1).

N^1-{3-(m-Tolyloxy)propyl}-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propyl-amino](amino)methylene}decanediamide (5.65)

The title compound was prepared from **5.65a** (20 mg, 0.027 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.65** as colorless oil (11 mg, 75 %). ^{1}H-NMR (CD_3OD) δ (ppm): 7.11 (t, ^3J =8.0 Hz, 1H, Ar-***H***), 6.71 (m, 2H, Ar-***H***), 6.67 (m, 1H, Ar-***H***), 3.97 (t, ^3J = 6.2 Hz, 2H, OC***H***$_2$CH$_2$), 3.35 (m, 4H, C***H***$_2$NH), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.45 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$), 2.28 (s, 3H, Ar-C***H***$_3$), 2.11 (m, 5H, Thiaz-4-C***H***$_3$, NHCOC***H***$_2$), 1.92 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$, OCH$_2$C***H***$_2$), 1.61 (m, 4H, COCH$_2$C***H***$_2$), 1.31 (m, 8H, (C***H***$_2$)$_4$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.39 (quat. ***C***=O), 160.18 (quat. Ar-***C***), 132.66 (quat. Thiaz-4-***C***), 122.53 (+, Ar-***C***H), 118.45 (quat. Thiaz-5-***C***), 116.28 (+, Ar-***C***H), 112.49 (+, Ar-***C***H), 66.43 (-, ***C***H$_2$-OAr), 41.64 (-, ***C***H$_2$NH), 37.77 (-, CONH***C***H$_2$), 37.54 (-, CO***C***H$_2$), 37.13 (-, CO***C***H$_2$), 30.18 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.93 (-, (***C***H$_2$)$_4$), 27.01 (-, ***C***H$_2$CH$_2$O), 25.40 (-, COCH$_2$***C***H$_2$), 23.62 (-, Thiaz-5-***C***H$_2$), 21.61 (+, Ar-***C***H$_3$), 11.48 (+, Thiaz-4-***C***H$_3$); HREIMS: *m/z* for ([$C_{28}H_{44}N_6O_3S$]$^{+\bullet}$) calcd. 544.3196, found 544.3181; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 3.88 (t_R = 13.06 min, column B), purity = 99 %; $C_{28}H_{44}N_6O_3S$ · 2TFA (772.79).

N^1-(6-[3,4-Dioxo-2-{3-[3-(piperidin-1-ylmethyl)phenoxy]propylamino}cyclobut-1-enylamino]hexyl)-N^{10}-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)-methylene}decanediamide (5.66)

The title compound was prepared from **5.66a** (25 mg, 0.024 mmol) in 5 ml DCM/abs and 1 ml TFA according to the general procedure yielding **5.66** as brown oil (10 mg, 50 %). EI-MS (70 eV) m/z (%): 411.9 ((M+2H)$^{2+}$, 100), 822.7 (MH$^+$, 10); HRLSIMS: *m/z* for ([$C_{43}H_{67}N_9O_5S$ + H]$^+$) calcd. 822.5059, found 822.5052; prep HPLC: MeCN/0.1 % TFA/aq (20/80-50/50); anal. HPLC: k`= 2.66 (t_R = 9.79 min, column B), purity = 96 %; $C_{43}H_{67}N_9O_5S$ · 3TFA (1164.17).

5.5.1.10 Preparation of the bivalent acylguanidines 5.56-5.58

General procedure for the synthesis of propionylated bivalent acylguanidines 5.56 and 5.57

NEt_3 (4 or 5 eq) was added to a solution of **5.41** or **5.54** (1 eq) in MeCN. Subsequently, a solution of NHS-propionate (0.8 eq) was added and stirred for 16 h at room temperature. The solvent was evaporated and the product purified by preparative RP-HPLC.

***N*^{1}-{[3-(2-Amino-4-methylthiazol-5-yl)propylamino](amino)methylene}-*N*^{10}-{[3-propionamidopropylamino](amino)methylene}decanediamide (5.56)**

The title compound was prepared from **5.41** (5.6 mg, 5.9 µmol) in 1.5 ml MeCN, NEt_3 (3.3 µl, 23.6 µmol) and NHS-propionate (0.8 mg, 4.7 µmol) in 0.5 ml MeCN according to the general procedure yielding **5.56** (2.8 mg, 86 %) as yellow oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.46 (m, 2H, C***H***$_2$NH), 3.36 (m, 2H, C***H***$_2$NH), 3.21 (m, 2H, C***H***$_2$NHCO), 2.71 (t, ^3J = 7.6 Hz, 2H, Thiaz-5-C***H***$_2$), 2.47 (t, ^3J = 7.4 Hz, 4H, COC***H***$_2$), 2.21 (m, 2H, C***H***$_2$CH$_3$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 2.04-1.74 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$, C***H***$_2$CH$_2$NH), 1.66 (m, 4H, COCH$_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$), 1.12 (t, ^3J = 7.6 Hz, 3H, CH$_2$C***H***$_3$); HREIMS: *m/z* for ([$C_{25}H_{45}N_9O_3S$ + H]$^+$) calcd. 552.3439, found 552.3438; prep HPLC: MeCN/0.1 % TFA/aq (10/90-40/60); anal. HPLC: k`= 2.01 (t_R = 8.05 min, column B), purity = 84 %; $C_{25}H_{45}N_9O_3S$ · 3TFA (893.81).

***N*^{1},*N*^{17}-Bis({3-[2-amino-4-methylthiazol-5-yl]propylamino}(amino)methylene)-9-[2-propionylaminoethyl]-5,13-dioxo-6,9,12-triazaheptadecanediamide (5.57)**

The title compound was prepared from **5.54** (8.5 mg, 6.4 µmol) in 0.8 ml MeCN, NEt_3 (4.5 µl, 32.5 µmol) and NHS-propionate (0.9 mg, 5.1 µmol) in 0.5 ml MeCN according to the general procedure yielding **5.57** (4.4 mg, 68 %) as brown oil. ^{1}H-NMR (CD_3OD) δ (ppm): 3.54 (m, 4H, CONHC***H***$_2$), 3.39 (m, 4H, C***H***$_2$NH), 3.23 (m, 4H, NHC***H***$_2$C***H***$_2$NH$_2$), 3.07 (m, 4H, CONHCH$_2$C***H***$_2$), 2.71 (t, ^3J = 7.4 Hz, 4H, Thiaz-5-C***H***$_2$), 2.54 (m, 4H, COC***H***$_2$), 2.34 (t, ^3J = 7.3 Hz, 4H, NHCOC***H***$_2$), 2.26 (m, 2H, COC***H***$_2$CH$_3$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.92 (m, 8H, Thiaz-5-CH$_2$C***H***$_2$, COCH$_2$C***H***$_2$), 1.11 (t, ^3J = 7.4 Hz, 3H, CH$_2$C***H***$_3$); HREIMS: *m/z* for ([$C_{35}H_{60}N_{14}O_2S_2$ + H]$^+$) calcd. 821.4385, found 821.4391; prep HPLC: MeCN/0.1 % TFA/aq (15/85-40/60); anal. HPLC: k`= 0.85 (t_R = 4.96 min, column B), purity = 81 %; $C_{35}H_{60}N_{14}O_2S_2$ · 4TFA (1277.15).

{13-Amino-3-[2-(5-{[3-(2-amino-4-methylthiazol-5-yl)propylamino](amino)-methylene}amino-5-oxopentanamido)ethyl]-17-(2-amino-4-methylthiazol-5-yl)-7,11-dioxo-3,6,12,14-tetraazaheptadec-12-enyl}-4-{(E)-2-(1,2,3,5,6,7-hexahydropyrido-[3,2,1-ij]quinolin-9-yl)vinyl}-2,6-dimethylpyridinium trifluoroacetate (5.58)

NEt_3 (2.7 µl, 19.6 µmol) was added to a solution of **5.54** (5.2 mg, 3.9 µmol) in 800 µl MeCN. Subsequently, a solution of py-1[23] ((E)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-9-yl)ethenyl]-2,6-dimethylpyrylium tetrafluoroborate, 0.6 mg, 1.6 µmol) in 50 µl DMF and 150 µl MeCN was added. After 1-2 min the color changed from blue to red. The reaction was stopped by addition of 10 % TFA/aq (30 µl) after an incubation period of 1 h at room temperature. The product was purified by preparative RP-HPLC (MeCN/0.1 % TFA/aq (30/70-70/30)) yielding **5.58** as red oil (1.1 mg, 69 %). EI-MS (70 eV) m/z (%): 526.8 ($(M^+ + H)^{2+}$, 70), 1052.8 (M^+, 10); anal. HPLC: k`= 1.81 (t_R = 7.52 min, column B), purity = 95 %; $C_{53}H_{78}N_{15}O_4S_2 \cdot 5TFA$ (1623.51).

5.5.2 Pharmacological methods

5.5.2.1 Materials

See section 3.5.2.1

5.5.2.2 Determination of histamine receptor agonism and antagonism in GTPase assays

See section 4.5.2.2

5.6 References

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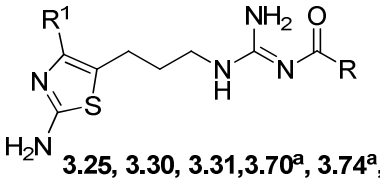
Chapter 6

Bioanalytical and toxicological investigations of representative acylguanidine-type histamine H_2R agonists

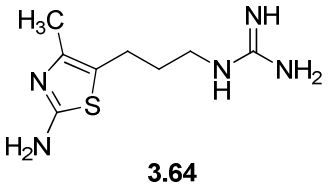
6.1 Introduction

As a prerequisite for the application of acylguanidine-type H_2R agonists as pharmacological tools in cell based *in vitro* studies or in future *in vivo* experiments, selected compounds were investigated regarding their drug-like properties and toxic effects. Monovalent and bivalent acylguanidine-type H_2R agonists presented in this work (cf. chapters 3-5) are of cationic amphiphilic nature due to their polar basic pharmacophoric groups (hetarylpropylguanidines) and their lipophilic fragments (spacer, alkyl and aryl residues, respectively). Given that amphiphilic substances such as surfactants are known to have a potential to interact with biological membranes, eventually resulting in membrane disruption and solubilization,[1] representative compounds were investigated with respect to their ability to induce the rupture of erythrocytes (hemolysis). In addition to the pharmacokinetic properties of compounds, many diverse mechanisms, like the formation of active metabolic intermediates or the interaction with off-targets, can impede the normal function of the cell and trigger cell death. Thus, selected compounds were investigated with respect to potential cytotoxic effects. In addition, the extent of plasma protein binding was studied. Tables 6.1 and 6.2 give an overview of selected monovalent and bivalent acylguanidine-type H_2R agonists.

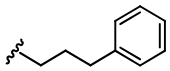
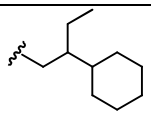
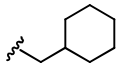
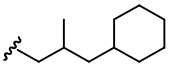
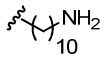
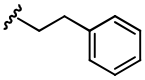
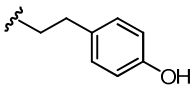
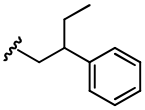
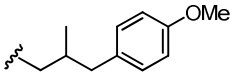
Table 6.1. Structures of investigated monovalent aminothiazolylpropylguanidines.



3.25, 3.30, 3.31,3.70[a], 3.74[a], 3.77[a], 3.78[a],3.80[a], 3.82[a]

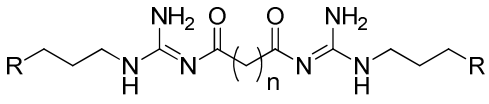


3.64

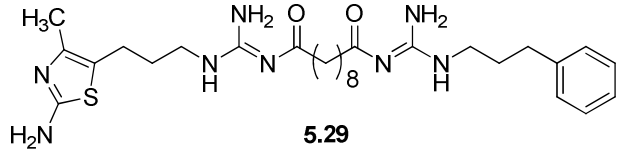
Compd.	R^1	R	Compd.	R^1	R
3.25	CH_3		**3.77**	CH_3	
3.30	CH_3		**3.78**	CH_3	
3.31	CH_3		**3.80**	H	
3.70	CH_3		**3.82**	H	
3.74	CH_3				

[a] Compounds **3.70**, **3.74**, **3.77**, **3.78**, **3.80** and **3.82** were provided by Dr. A. Kraus.[2]

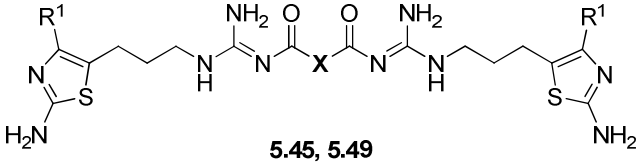
Table 6.2. Structures of investigated bivalent hetarylpropylguanidines.



4.10, 4.18, 4.19, 4.27[a], 4.30[a]



5.29



5.45, 5.49

Compd.	n	R	Compd.	n	R
4.10	6	2-aminothiazol-5-yl	**4.30[a]**	20	2-amino-4-methylthiazol-5-yl
4.18	14	imidazol-4-yl	**Compd.**	**R^1**	**X**
4.19	20	imidazol-4-yl	**5.45**	CH_3	CH_2-Ph-CH_2
4.27[a]	8	2-amino-4-methylthiazol-5-yl	**5.49**	H	$(CH_2)_3$-S-S-$(CH_2)_3$

[a] Compounds **4.27** and **4.30** were provided by Dr. A. Kraus.[2]

6.2 Results and discussion

6.2.1 Hemolytic properties of selected acylguanidine-type H_2R agonists

The red blood cell is a very commonly used model for studies of amphiphilic drugs. It is well known that interaction of amphiphilic substances with the erythrocyte membrane can lead to hemolysis by inducing osmotic pressure followed by cell swelling or by partial solubilization of membrane lipids and proteins, e.g. by formation of mixed micelles.[4] Hemolysis means the abnormal breakdown of red blood cells, which leads to the release of hemoglobin from erythrocytes. Due to the characteristic absorption maximum of hemoglobin at 580 nm, the hemolytic effect of the investigated title compounds was determined spectrophotometrically. In Figure 6.1 the percentage of hemolysis induced by the investigated compounds (**3.25**, **3.30**, **3.31**, **3.64**, **3.77**, **3.78**, **3.80**, **4.10**, **4.18**, **4.19**, **4.27**, **4.30**, **5.29**, **5.45** and **5.49**) is shown compared to the reference compound digitonin, which is known to induce strong hemolysis.[3]

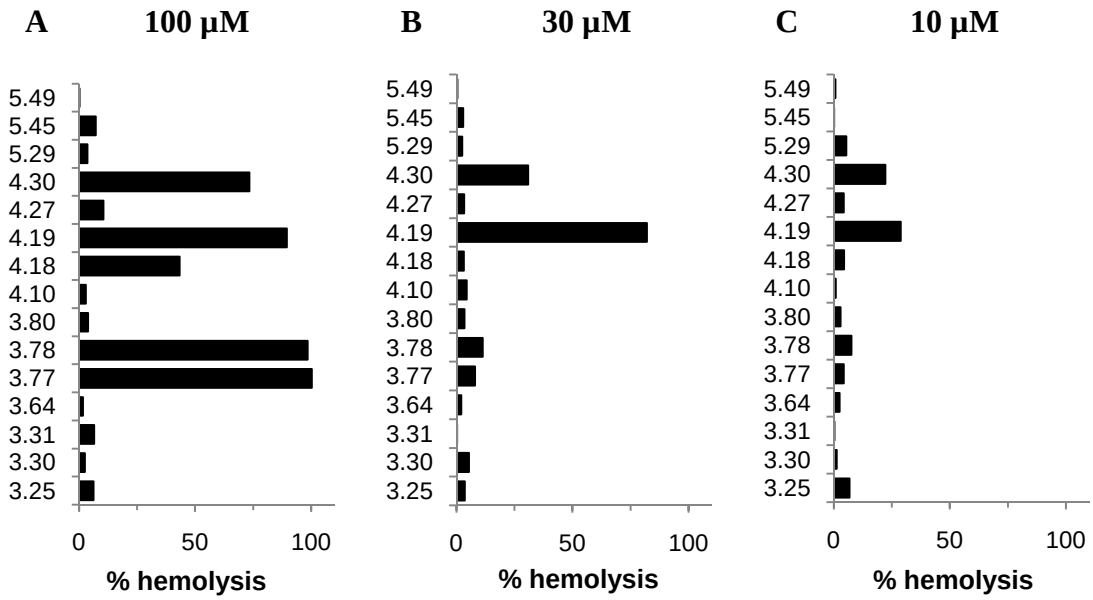


Figure 6.1. Percentage of hemolysis induced by selected N^G-acylated hetarylpropylguanidines at 100 µM (**A**), 30 µM (**B**) and 10 µM (**C**) compared to the reference compound digitonin.

The hemolytic activity of bivalent acylguanidine-type H_2R agonists was strongly dependent on the spacer length. While moderate (20-40 %) to severe (70-100 %) hemolysis had been observed in the concentration range of 10-100 µM for compounds with long lipophilic alkanediyl spacers (**4.18**, **4.19** and **4.30**, cf. Table 6.2 for structures, n

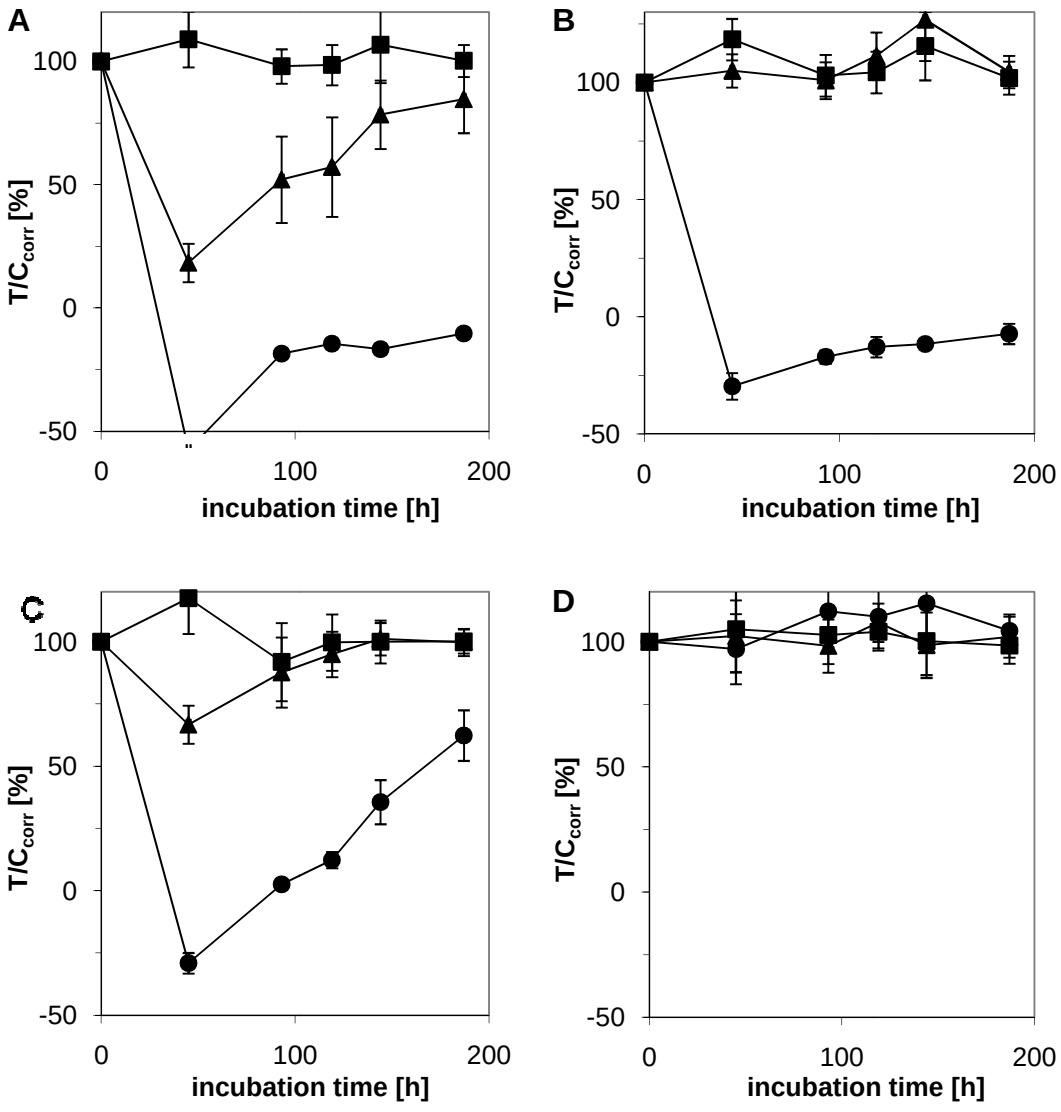
≥ 14), the shorter bivalent ligands were essentially devoid of hemolytic activity. Concerning monovalent ligands, severe hemolysis was induced by **3.77** and **3.78** at a concentration of 100 µM. All other monovalent compounds revealed only minor hemolytic activity. Notably, **3.77** and **3.78** had a cyclohexyl residue in common. Highly lipophilic moieties, especially cyclohexyl and long lipophilic polymethylene linkers, give a critical amphiphilic character, resulting in severe cell-damaging effects of such substances. By contrast, compounds bearing less lipophilic residues had a decreased or negligible tendency for solubilization of cell membranes. With exception of bivalent ligands comprising a 20-membered carbon spacer (**4.19** and **4.30**), the hemolytic effect of all investigated compounds was essentially marginal (< 7 %) at concentrations as low as 30 µM. Probably, the used concentrations of these compounds were below the critical micellar concentration to effectively damage the erythrocyte membrane. Compounds having less "tenside-like" character were found to be devoid of hemolytic activity up to concentrations as high as 100 µM. In conclusion, with respect to cellular *in vitro* investigations or *in vivo* experiments, concentrations higher than 30 µM of amphiphilic N^G-acylated hetarylpropylguanidines, especially those bearing highly lipophilic residues, should be avoided.

6.2.2 Cytotoxicity of selected acylguanidine-type H_2R agonists in the crystal violet based chemosensitivity assay

The cytotoxic properties of selected acylguanidine-type H_2R agonists (**3.25**, **3.30**, **3.80**, **4.10**, **4.19**, **4.27**, **4.30**, **5.29** and **5.49**) were studied in a kinetic crystal violet based chemosensitivity assay over a period of approximately 200 h using proliferating human HT-29 colon carcinoma cells.[5] Cisplatin was taken as reference compound. Figure 6.2 shows the cytotoxic effects as plots of corrected T/C values versus time of incubation.

In accordance to the results from the hemolysis studies, the cytotoxic effect of bivalent ligands was strongly dependent on the spacer length. Only compounds **4.19** and **4.30**, comprising long lipophilic polymethylene spacers, showed strong cytotoxic effects. For all other investigated bivalent acylguanidines cell proliferation was not affected up to a concentration of 30 µM. Concerning monovalent acylguanidines, all three tested compounds showed more or less distinct cytotoxicity in the concentration range of 10-30 µM. These observations differed from the results of the hemolysis studies, in which **3.25**,

3.30 and **3.80** produced only marginal cell-damaging effects. Notably, after approximately 200 h cytocidal effects (T/C_{corr} < 0 %) were detected for 3-(2-amino-4-methylthiazol-5-yl)propylguanidines with phenylalkanoyl (**3.25**, Figure 6.2 **A**) and cyclohexylalkanoyl residues (**3.30**, Figure 6.2 **B**), respectively, whereas the cell population treated with **3.80** (Figure 6.2 **C**), recovered after initial damage (cytotoxic effect, T/C_{corr} > 0 %). Compared to the investigated monovalent acylguanidines **3.25**, **3.30** and **3.80** (log $D_{[7.4]}$ = 1.7-3.9), bivalent compounds **4.27**, **5.29** and **5.49** with comparable overall lipophilicity (log $D_{[7.4]}$ = 2.1-3.7) showed significantly reduced cytotoxic effects. Thus, the linkage of two pharmacophoric moieties through spacers of appropriate length is beneficial with respect to cytotoxicity, presumably due to reduced amphiphilic character. In summary, in view of future cell based *in vitro* investigations or *in vivo* experiments, potential cytotoxic effects of acylguanidine-type H_2R agonists at micromolar concentrations should be taken into account.



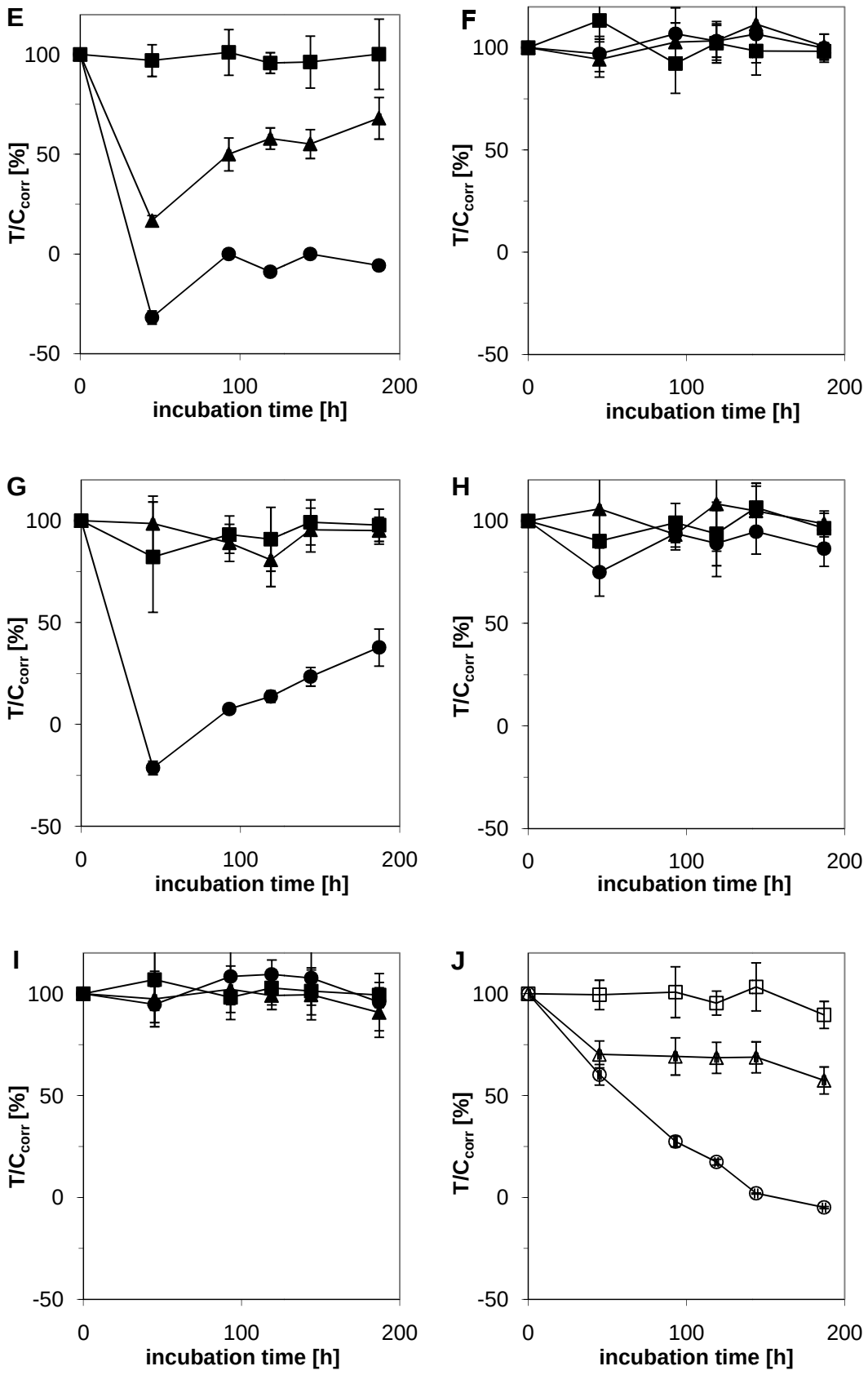


Figure 6.2. Effects of selected acylguanidine-type H_2R agonists on proliferating HT-29 cells upon long-term exposure. Investigated compounds: **3.25** (**A**), **3.30** (**B**), **3.80** (**C**), **4.10** (**D**), **4.19** (**E**), **4.27** (**F**), **4.30** (**G**),

5.29 (**H**) and **5.49** (**I**). Following concentrations were used: 3 µM (■), 10 µM (▲) and 30 µM (●). Cisplatin (**J**) was used as positive control at following concentrations: 0.3 µM (□), 1 µM (Δ) and 3 µM (○).

6.2.3 Investigations on plasma protein binding

The efficiency of drugs is affected by the degree to which they bind within blood plasma. Serum albumin is the most abundant protein in mammalian plasma. To investigate the protein binding of selected acylguanidines (**3.25**, **3.70**, **3.74**, **3.78**, **3.80**, **3.82**, **4.18**, **4.19**, **4.27**, **4.30**, **5.29** and **5.45**) an HPLC method was applied using bovine serum albumin (BSA) as protein component. After incubation for 1 h and filtration with a cutoff of 10 kDa to remove serum albumin, samples before filtration, samples from supernatant and samples from filtrate were analyzed. Examples of HPLC-traces are depicted in Figure 6.3. In the control experiments (without BSA), the investigated compounds were able to pass the membrane to an average extent of 85 % (cf. Figure 6.3 **F**). This value was considered in the calculation of the protein binding (Table 6.3). With exception of **3.70** (63 %), **3.80** (79 %) and **3.82** (87 %), all investigated mono- and bivalent compounds were nearly completely bound to serum albumin (90-99 %). Notably, **3.80** and **3.82**, which are lacking the 4-methyl group at the aminothiazole ring, and **3.70**, which has an additional hydroxyl group at the phenyl ring, are the compounds with lowest lipophilicity (log $D_{[7.4]}$ = 1.7-2.7) among the investigated H_2R agonists. In summary, the synthesized compounds revealed a high degree of plasma protein binding. This must be taken into account, when acylguanidines are investigated *in vivo*.

Table 6.3. Percentage of protein binding for representative acylguanidines.

Compd.	PPB[%]	Compd.	PPB[%]
3.25	90	**4.18**	98
3.70	63	**4.19**	98
3.74	90	**4.27**	91
3.78	97	**4.30**	95
3.80	79	**5.29**	98
3.82	87	**5.45**	99

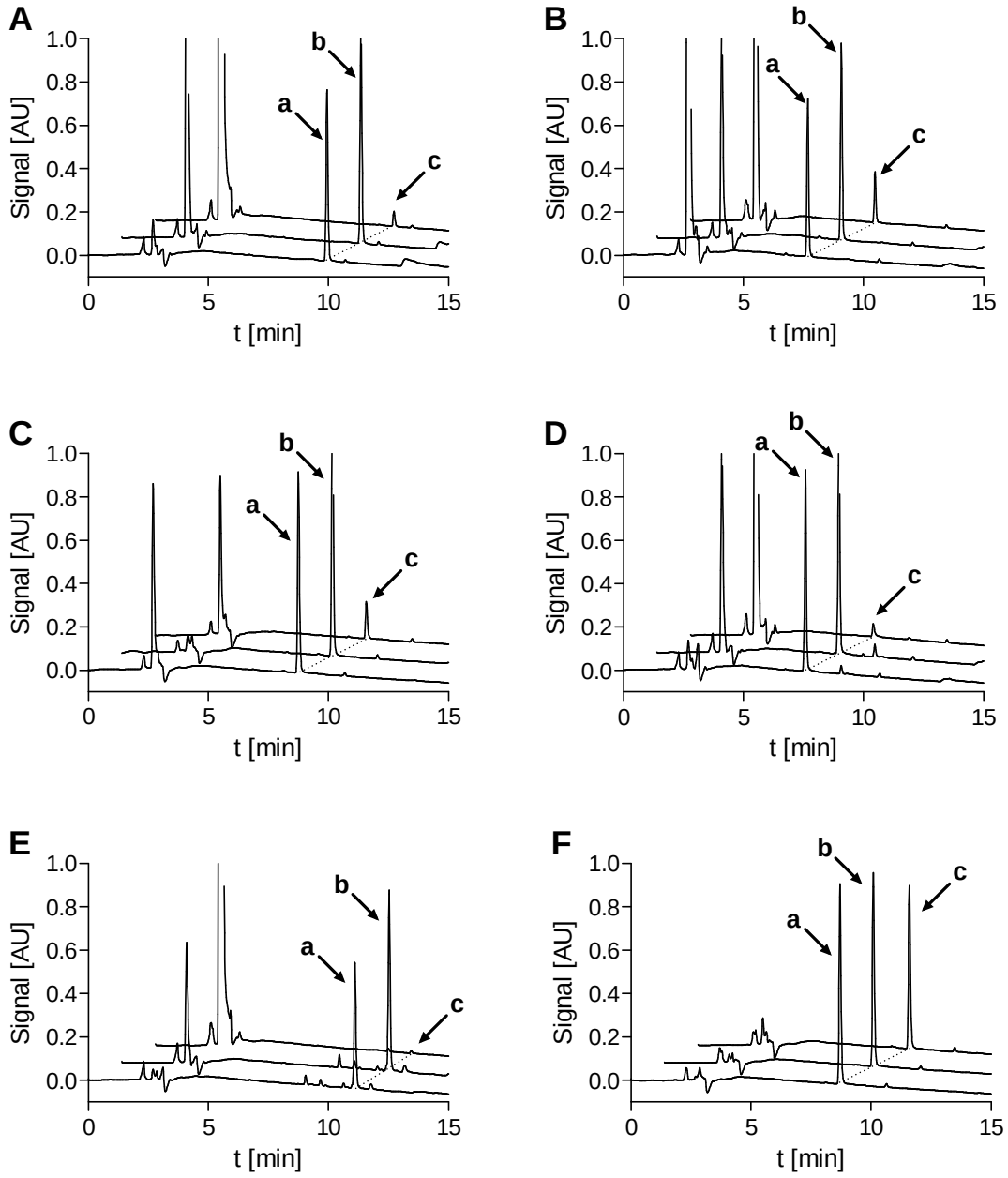


Figure 6.3. HPLC traces of samples containing **3.25** (**A**), **3.70** (**B**), **3.80** (**C**), **4.27** (**D**) and **5.29** (**E**) in presence of bovine serum albumin taken **a**) before filtration, **b**) from the supernatant and **c**) from the filtrate. In control experiments (without BSA) the compounds were able to pass the membrane to an average extent of 85 %, as shown for **3.80** as an example (**F**).

6.3 Summary

To characterize acylguanidine-type H_2R agonists with respect to their use in cell based *in vitro* studies or future *in vivo* experiments, selected compounds were investigated

regarding their hemolytic and cytotoxic properties as well as their potential to bind to plasma proteins. Among all investigated compounds, strong hemolytic effects were only induced by compounds with most distinct amphiphilic properties due to highly lipophilic structural moieties, such as cyclohexyl residues or long polymethylene spacers in case of monovalent and bivalent ligands, respectively. All other compounds were found to induce only minor hemolytic effects up to concentrations as high as 100 µM. Obviously, the increase in lipophilicity and in overall amphiphilicity led to enhanced damage of the erythrocyte membrane, presumably through the formation of mixed micelles. In accordance to the results from the hemolysis studies, the cytotoxic effects of bivalent ligands were strongly dependent on the spacer length. Only compounds **4.19** and **4.30**, comprising the longest lipophilic polymethylene spacers, showed strong cytotoxic effects. Concerning monovalent ligands, the results from the crystal violet based chemosensitivity assay and from hemolysis studies differed: all tested compounds showed distinct cytotoxic or cytocidal effects at a concentration of 30 µM, regardless of negligible hemolytic activities. Adverse effects probably resulted from intracellular toxic effects of compounds. In so far, the predictive value of the hemolysis assay is limited, regardless of the tenside-like character of the considered compounds. However, interactions with cell membranes (e.g. hemolytic effect) also reflect physicochemical properties, which might play a role in cellular uptake and binding to various off-targets. Furthermore, the investigated compounds revealed a high degree of plasma protein binding. In summary, these results must be taken into account with respect to the application of acylguanidine-type H_2R agonists as pharmacological tools to perform *in vivo* or cell based *in vitro* studies. The drug-like properties of these H_2R agonists should be further improved.

6.4 Experimental section

6.4.1 Determination of hemolytic properties of acylguanidine-type H_2R agonists using human erythrocytes

Isotonic saline (2 ml) was added to fresh citrated human blood (1 ml) and the suspension was centrifuged at 4 °C (70 g, 15 min). After removal of the supernatant plasma and the leukocyte-layer, the erythrocytes were re-suspended in isotonic saline (1 ml) and centrifuged again (2000 g, 10 min, 4 °C). The supernatant was discarded, the washing

procedure was repeated twice and the erythrocytes were stored on ice before used on the same day. Stock solutions (1.5 mM and 5 mM) of the test compounds, dissolved in 70 % EtOH, were prepared. 500 µl of freshly prepared erythrocytes were diluted with 9.5 ml of isotonic saline. Subsequently, aliquots of 50 µl were filled into each well of a microtiter plate (Greiner, Frickenhausen, Germany) and 1 µl of respective test compound stock solutions was added to obtain the final concentration of the test compounds (30 µM and 100 µM). For the negative control (0 % hemolysis) 1 µL of 70 % EtOH was added, and to achieve 100 % hemolysis 1 µl of digitonin solution (2 %, w/v) was used as reference. Each sample was prepared in duplicate. After careful mixing, the microtiter plate was incubated for 1 h at 37 °C and vortexed every 20 min. Thereafter, the plate was centrifuged at 2000 g for 3 min. 30 µl of each well were transferred to a new microtiter plate and 100 µl of isotonic saline were added into each well. The absorbance was measured at 580 nm ($\lambda_{\text{hemoglobin, max}}$) and at 485 nm ($\lambda_{\text{hemoglobin, min}}$) using a GENios Pro microplate reader (Tecan Deutschland GmbH, Crailsheim, Germany). The hemolytic activity (percentage) was calculated according to

$$\%\ \text{Hemolysis} = \left(\frac{A_{580\ \text{nm}}}{A_{485\ \text{nm}}} - \frac{A_{580\ \text{nm (0 \%)}}}{A_{485\ \text{nm (0 \%)}}}\right) \Big/ \left(\frac{A_{580\ \text{nm (100 \%)}}}{A_{485\ \text{nm (100 \%)}}} - \frac{A_{580\ \text{nm (0 \%)}}}{A_{485\ \text{nm (0 \%)}}}\right) \cdot 100$$

where $A_{580\ \text{nm}}$ and $A_{485\ \text{nm}}$ are the measured absorbances of the sample at 580 nm and 485 nm, respectively, $A_{580\ \text{nm (0 \%)}}$ and $A_{485\ \text{nm (0 \%)}}$ are the measured absorbances of the control at 580 nm and 485 nm, respectively, and $A_{580\ \text{nm (100 \%)}}$ and $A_{485\ \text{nm (100 \%)}}$ are the measured absorbances of the reference containing digitonin at 580 nm and 485 nm, respectively.

6.4.2 Crystal violet based chemosensitivity assay using proliferating human HT-29 colon carcinoma cells

The assay was performed as previously described.[5] Accordingly, tumor cells were seeded into flat-bottomed 96-well plates (Greiner, Frickenhausen, Germany) at a density of approximately 15 cells per microscopic field (magnification: 320-fold). After 2 to 3 days of incubation (37 °C, 5 % CO_2), the culture medium was removed by suction and replaced by fresh medium containing the test compounds at various concentrations. Cells treated with medium containing the respective solvent used for the test compounds served as control. After various incubation periods the cells were fixed with 1 % glutardialdehyde solution in PBS and stored at 4 °C. At the end of the experiment all

plates were stained with crystal violet (Serva, Heidelberg, Germany) simultaneously. Subsequently, excess dye was removed with water and cell-bound crystal violet was re-dissolved with 70 % EtOH. The absorbance was measured at 580 nm using a GENios Pro microplate reader (Tecan Deutschland GmbH, Crailsheim, Germany). The effects of the test compounds on the proliferating cells were presented as corrected T/C values according to

$$T/C_{corr}\ (\%) = \frac{(T - C_0)}{(C - C_0)} \cdot 100$$

where T = mean absorbance of treated cells, C = mean absorbance of controls, C_0 = mean absorbance at the time when test compounds were added (t = 0).

6.4.3 Determination of protein binding using HPLC

Freshly prepared stock solutions of the test compounds (15 µl, 10 mM) were added to a solution of BSA (485 µl, 600 µM) and the mixtures were incubated for 1 h at 37 °C. 400 µl of the incubation mixture were filtered using Nanosep centrifugal filter devices (10K Omega, 10000 MWCO, Pall Life Science, New York, USA). After filtration of approximately half of the solution (13000 g, 1-3 min), 100 µl samples of the filtrate, the supernatant and the unfiltered sample were taken and diluted with 200 µl of ice-cold MeCN. The solutions were stored in the refrigerator for 30 min to complete deproteinization and centrifuged at 4 °C (13000 g, 5 min). 200 µl of the supernatant were transferred into new vials and the solvent removed under reduced pressure. The residues were dissolved in 300 µl of MeCN/TFA (0.05 % aq) (10/90) and used for HPLC analysis immediately. As a control, BSA was replaced by phosphate buffer (pH 7.4) and the same procedure was repeated.

Analytical HPLC was performed on a system from Thermo Separation Products equipped with an SN400 controller, P4000 pump, an AS3000 autosampler, and a Spectra Focus UV/Vis detector. Stationary phase was a Nucleodur-C_{18}HTec (250 x 4.0, 5µM) column (Macherey-Nagel, Düren, Germany) thermostated at 30°C. As mobile phase, gradients of MeCN/TFA (0.05 % aq) were used (flow rate = 0.75 ml·min^{-1}). Gradient mode: 0 min: MeCN/TFA (0.05% aq) 10:90, 20 min: 60:40, 21 min: 95:5, -29 min: 95:5. Absorbance was detected at 210 nm. The percentage of plasma protein binding (PPB) was calculated according to

$$\text{PPB}\ (\%) = 100 - \frac{1.15 * \text{A}_{\text{filtrate}}}{\text{A}_{\text{unfiltered}}} \cdot 100$$

where $A_{filtrate}$ is the peak area under the curve of the filtrate sample, $A_{unfiltered}$ is the peak area under the curve of the sample before filtration and factor 1.15 represents the mean impermeability of the centrifugal filter devices determined for control samples without BSA.

6.5 References

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Chapter 7

Summary

Potent and selective histamine H_2 receptor (H_2R) agonists, including brain-penetrating compounds, are required as pharmacological tools to evaluate the (patho)physiological role of H_2Rs. Moreover, H_2R agonists might be of therapeutic value as drugs, for example, in the treatment of acute myelogenous leukemia.

Previously, acylguanidine-type H_2R agonists with reduced basicity were synthesized in our laboratory, resulting in improved bioavailability and CNS penetration compared to the corresponding guanidines. Based on the preceding work, this thesis aimed at the design, the synthesis and the pharmacological characterization of novel N^G-acylated hetarylpropylguanidines to elaborate the structure-activity relationships (SAR) in more detail. A central aspect of this project was the development of bivalent acylguanidine-type H_2R agonists.

The prepared compounds were investigated for H_2R agonism in GTPase and [^{35}S]GTPγS binding assays at guinea pig (gp) and human (h) H_2R-$Gs\alpha_S$ fusion proteins including various H_2R mutants, at the isolated gp right atrium (in cooperation with Prof. Elz, University of Regensburg), and, with respect to H_2R selectivity, in GTPase assays for activity on recombinant human H_1, H_3 and H_4 receptors. In addition, representative compounds were investigated regarding their hemolytic and cytotoxic properties as well as their potential to bind to plasma proteins.

N^G-Acylated 3-(2-aminothiazol-5-yl)propylguanidines proved to be H_2R partial to full agonists. Within this series, highest potencies resided in compounds having a two- or three-membered carbon chain between carbonyl group and phenyl or cyclohexyl ring, respectively. Notably, the introduction of a free amino group at an appropriate distance to the pharmacophoric moiety was beneficial with respect to H_2R agonistic potency. In

contrast to their imidazole analogs, the aminothiazoles were highly selective for the H_2R *vs.* other HR subtypes. Thus, this study substantiates previous results, confirming that the 2-aminothiazole and the imidazole moiety are bioisosteric groups at the H_2R but not at the H_3R and H_4R. Moreover, in contrast to amthamine, the 4-methyl group at the thiazole ring did not significantly contribute to the H_2R agonism of N^G-acylated 3-(2-amino-4-methylthiazol-5-yl)propylguanidines.

Bivalent H_2R agonists were synthesized by connecting the guanidine groups of two molecules by N^G-acylation with dicarboxylic acids of different structure and length (spacer lengths ≈ 6 – 27 Å). The bivalent ligands proved to be up to two orders of magnitude more potent than monovalent acylguanidines and up to 4000 times more potent than histamine at the gpH_2R (compounds with octanedioyl to decanedioyl spacers). These are the most potent histamine H_2R agonists known to date. However, due to insufficient spacer lengths of the most active compounds, the tremendous gain in potency compared to monovalent analogs cannot be explained by simultaneous occupation of the orthosteric recognition sites of a H_2R dimer. The high potency rather results from interaction with an accessory (allosteric?) binding site at the same receptor protomer.

Replacing the second hetarylpropylguanidine moiety with simple alkyl guanidine groups afforded rather high H_2R agonistic activities (EC_{50} values in the low nanomolar range), whereas all other variations in this part of the molecule led to drastically decreased potencies. A further decrease in potency resulted from the elimination of the second guanidino group, corroborating the importance of a basic centre at an appropriate distance to the pharmacophore to obtain highly potent bivalent H_2R agonists. These results are consistent with the concept of interaction with the orthosteric and an accessory binding site of one H_2R protomer, i. e. the accessory binding site can accommodate the second acylguanidine portion.

All investigated compounds were significantly more potent and efficacious at the gpH_2R relative to the hH_2R. These differences might help to verify the suggested model of bivalent ligand - receptor interactions via identification of species-dependent molecular determinants of the orthosteric and the accessory binding site in hH_2R and gpH_2R, respectively. Investigations on gpH_2R and hH_2R mutants/chimera confirmed the key role of non-conserved Tyr-17 and Asp-271 in TM1 and TM7 in the gpH_2R for species-selective H_2R activation and suggested that the e2 loop does not participate in direct

ligand - receptor interaction. To explore the topology of this putative accessory binding site in more detail, further studies on H_2R mutants are necessary.

In conclusion, bioisosteric and bivalent approaches applied in this thesis led to highly potent and selective pharmacological tools for more detailed investigations of the H_2R. However, in view of cell based *in vitro* investigations or future *in vivo* experiments, the drug-like properties of these H_2R agonists should be further improved.

Chapter 8

Appendix

8.1 HPLC purity data

Cmpd.	t_R (min)	k´	purity (%)	Cmpd.	t_R (min)	k´	purity (%)
3.19[a]	4.62	0.73	99.0	**4.12**[b]	15.40	3.64	99.0
3.20[a]	7.91	2.96	96.4	**4.13**[b]	20.63	5.22	99.1
3.21[a]	13.54	4.06	96.2	**4.14**[b]	6.36	0.92	95.6
3.22[b]	9.95	2.00	97.7	**4.15**[b]	7.01	1.11	89.7
3.23[b]	10.79	2.25	99.7	**4.16**[b]	7.81	1.35	95.0
3.24[b]	11.23	2.39	98.6	**4.17**[b]	8.74	1.63	90.2
3.25[b]	12.83	2.87	99.5	**4.18**[b]	14.75	3.44	99.3
3.26[b]	14.06	3.24	99.4	**4.19**[b]	19.61	4.91	95.9
3.27[b]	15.21	3.58	95.0	**4.20**[a]	4.09	0.53	100
3.28[b]	14.81	3.46	100	**4.21**[a]	6.48	1.42	90.9
3.29[b]	11.34	2.24	97.9	**4.22**[a]	15.57	4.39	96.9
3.30[b]	12.77	2.85	97.7	**4.24**[a]	7.41	1.77	100
3.31[a]	7.89	1.94	96.1	**5.26**[b]	9.82	1.96	95.3
3.32[a]	4.31	0.61	100	**5.27**[b]	9.51	1.87	94.2
3.33[a]	7.34	1.74	100	**5.28**[a]	8.07	2.02	97.69
3.34[a]	13.29	3.97	99.6	**5.29**[b]	14.13	3.26	96.3
3.35[a]	23.53	7.80	92.6	**5.30**[a]	10.74	3.02	96.0
3.36[a]	9.60	2.95	98.9	**5.31**[a]	12.91	3.82	97.7
3.37[a]	11.44	3.28	99.5	**5.32**[a]	14.15	4.29	96.4
3.38[a]	13.31	3.97	100	**5.33**[b]	12.67	2.82	88.31
3.39[a]	9.98	2.73	99.5	**5.34**[b]	13.29	3.01	98.2
3.40[a]	10.89	3.07	99.5	**5.35**[b]	13.31	3.01	93.3
3.41[a]	11.09	3.14	99.4	**5.36**[a]	10.19	2.81	96.9
3.42[a]	6.80	1.54	100	**5.37**[a]	10.06	2.76	97.1
3.43[a]	5.46	1.04	96.7	**5.38**[a]	7.59	1.84	98.6

3.44[a]	10.96	3.10	99.4	**5.39**[a]	8.82	2.30	97.8
3.45[a]	11.09	3.15	99.5	**5.40**[a]	9.66	2.61	98.7
3.46[a]	10.99	3.11	99.7	**5.41**[a]	6.66	1.49	88.4
3.47[a]	10.13	2.79	99.5	**5.42**[a]	7.76	1.90	96.1
3.48[a]	11.60	3.34	99.1	**5.43**[a]	15.22	4.69	99.6
3.49[a]	12.46	3.66	100	**5.44**[b]	8.23	1.48	85.0
3.50[a]	15.48	4.77	99.1	**5.45**[a]	6.09	1.28	94.3
3.51[a]	13.78	4.15	99.0	**5.46**[a]	5.43	1.03	93.9
3.52[a]	14.63	4.47	99.8	**5.47**[b]	9.88	1.98	90.4
3.53[a]	3.42	0.28	97.9	**5.48**[a]	6.73	1.51	97.8
3.54[a]	7.56	1.83	100	**5.49**[a]	6.85	1.56	97.3
3.55[a]	8.49	2.17	96.8	**5.50**[a]	9.18	2.43	98.2
3.56[a]	9.30	2.48	98.5	**5.51**[a]	9.30	2.47	96.6
3.57[a]	12.02	3.49	99.2	**5.52**[a]	4.73	0.77	90.0
3.58[a]	15.15	4.66	97.4	**5.53**[a]	3.46	0.29	96.7
3.59[a]	11.21	3.19	98.20	**5.54**[a]	4.21	0.57	99.8
3.60[a]	15.84	4.92	95.8	**5.55**[a]	6.00	1.24	94.0
3.61[a]	8.43	2.15	96.5	**5.56**[a]	8.05	2.01	84.4
3.62[a]	5.84	1.18	84.8	**5.57**[a]	4.96	0.85	90.7
3.63[a]	8.90	2.33	100	**5.58**[a]	7.52	1.81	95.4
3.64[a]	3.41	0.27	94.8	**5.63**[a]	9.08	2.39	99.2
3.65[a]	3.40	0.27	100	**5.64**[a]	13.27	3.96	100
4.9[b]	10.22	2.08	92.2	**5.65**[a]	13.06	3.88	98.7
4.10[b]	8.66	1.61	94.6	**5.66**[a]	9.79	2.66	95.7
4.11[b]	10.37	2.13	100				

[a] TSP-system, gradient mode: MeCN/TFA (0.05% aq): 0 min: 10:90, 20 min: 60:40, 21 min: 95:5, -29 min: 95:5, flow rate = 0.75 mL min^{-1}, t_0 = 2.675 min; k`= $(t_R–t_0)/t_0$. [b] TSP-system, gradient mode: MeCN/TFA (0.05% aq): 0 min: 10:90, 20 min: 60:40, 23 min: 95:5, -33 min: 95:5, flow rate = 0.7 mL $min^{-1}$, $t_0$ = 3.318 min, k`= $(t_R–t_0)/t_0$.

8.2 Log D values at pH = 7.4

Cmpd.	log $D_{[7.4]}$[a]	Cmpd.	log $D_{[7.4]}$[a]	Cmpd.	log $D_{[7.4]}$[a]	Cmpd.	log $D_{[7.4]}$[a]
3.19	1.94	**3.51**	3.61	**3.83**	2.58	**5.32**	4.47
3.20	2.95	**3.52**	4.12	**3.84**	3.10	**5.33**	2.70
3.21	4.99	**3.53**	-2.95	**3.85**	3.61	**5.34**	3.82
3.22	2.55	**3.54**	-0.23	**4.9**	2.75	**5.35**	3.82
3.23	2.57	**3.55**	1.50	**4.10**	-1.10	**5.36**	3.21
3.24	3.13	**3.56**	1.43	**4.11**	-0.01	**5.37**	2.99

3.25	3.66	**3.57**	3.83	**4.12**	2.96	**5.38**	0.86
3.26	4.19	**3.58**	0.94	**4.13**	6.01	**5.39**	1.87
3.27	4.72	**3.59**	4.60	**4.14**	-0.41	**5.40**	2.29
3.28	4.26	**3.60**	2.07	**4.15**	-0.02	**5.41**	-0.72
3.29	3.67	**3.61**	0.83	**4.16**	0.49	**5.42**	3.45
3.30	3.91	**3.62**	1.29	**4.17**	1.00	**5.43**	9.42
3.31	1.45	**3.63**	1.87	**4.18**	4.56	**5.44**	3.56
3.32	0.27	**3.64**	-1.26	**4.19**	7.62	**5.45**	2.13
3.33	1.28	**3.65**	-2.94	**4.20**	-2.96	**5.46**	1.68
3.34	3.32	**3.66**	3.73	**4.21**	-2.12	**5.47**	4.82
3.35	8.41	**3.67**	4.24	**4.22**	5.01	**5.48**	-0.30
3.36	2.23	**3.68**	3.95	**4.24**	-0.83	**5.49**	0.06
3.37	2.76	**3.69**	4.16	**4.25**	1.45	**5.50**	1.35
3.38	3.23	**3.70**	2.72	**4.26**	2.25	**5.51**	2.07
3.39	2.05	**3.71**	0.01	**4.27**	3.25	**5.52**	1.40
3.40	2.56	**3.72**	4.26	**4.28**	4,27	**5.53**	-3.93
3.41	2.48	**3.73**	4.31	**4.29**	6.31	**5.54**	-3.80
3.42	1.04	**3.74**	4.15	**4.30**	9.36	**5.55**	6.10
3.43	-1.67	**3.75**	4.77	**4.31**	1.52	**5.56**	1.26
3.44	2.59	**3.76**	5.28	**4.32**	-1.31	**5.57**	-1.88
3.45	2.64	**3.77**	5.28	**5.26**	1.57	**5.58**	-0.42
3.46	2.48	**3.78**	5.28	**5.27**	1.42	**5.63**	3.25
3.47	2.23	**3.79**	5.79	**5.28**	1.20	**5.64**	6.31
3.48	2.74	**3.80**	1.70	**5.29**	3.69	**5.65**	4.94
3.49	3.0	**3.81**	1.81	**5.30**	4.05	**5.66**	3.86
3.50	4.47	**3.82**	2.32	**5.31**	7.03		

[a] Distribution coefficient at pH = 7.4, calculated with ACD/ChemSketch 12.0, Toronto, Canada.

8.3 Short lectures and poster presentations

"Synthesis and structure-activity relationships of bivalent acylguanidine-type histamine H_2 receptor agonists", short lecture in occasion of the 40th EHRS Meeting, Sochi, Russia, May 11 – 15, **2011**, Abstract published in: Inflamm. Res. (2011).

Birnkammer T., Kraus A., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Structure-activity relationships of bivalent acylguanidine-type histamine H_2 receptor

agonists", 5th Summer School Medicinal Chemistry, University of Regensburg, September 13 – 15, **2010**.

"Application of the bivalent ligand approach to acylguanidines resulted in highly potent and selective histamine H_2 receptor agonists", short lecture in occasion of the 39th EHRS Meeting, University of Durham (England), July 13 – 17, **2010**, Abstract published in: Inflamm. Res. (2010) 59 (Suppl 4): S305-S359.

"Bivalent Acylguanidines are Histamine H_2R Superagonists", short lecture in occasion of the Christmas Colloquium of the Department of Organic Chemistry, University of Regensburg, December 16, **2009**.

"Application of the bivalent ligand approach to acylguanidines: a route to histamine H_2 receptor superagonists", short lecture in occasion of the annual meeting "Internationale Doktorandentagung" of the German Pharmaceutical Society (DPhG), Pichlarn (Austria), November 19, **2009**.

Birnkammer T., Kraus A., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Bivalent acylguanidine-type ligands are highly potent and selective histamine H_2 receptor agonists", Annual meeting of the German Pharmaceutical Society (DPhG), University of Jena, September 29 – October 01, **2009**.

Elz S., Igel P., Geyer R., Kraus A., Kunze M., Birnkammer T., Buschauer A., "Cimetidine: a veteran H_2-receptor antagonist for the characterisation of novel potent acylguanidine-type H_2-receptor agonists", Annual meeting of the German Pharmaceutical Society (DPhG), University of Jena, September 29 – October 01, **2009**.

Lopuch M., Birnkammer T., Bernhardt G., Seifert R., Buschauer A., "Histamine H_2 receptor binding of potent mono- and bivalent acylguanidine-type agonists", Annual meeting of the German Pharmaceutical Society (DPhG), University of Jena, September 29 – October 01, **2009**.

Birnkammer T., Kraus A., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Toward bivalent acylguanidine-type ligands: highly potent and highly selective histamine H_2 receptor agonists", 38th EHRS Meeting, University of Fulda, May 13 – 16, **2009**.

Elz S., Igel P., Geyer R., Kraus A., Kunze M., Birnkammer T., Buschauer A., "Cimetidine: a veteran H_2-receptor antagonist for the characterisation of novel potent acylguanidine-type H_2-receptor agonists", 38th EHRS Meeting, University of Fulda, May 13 – 16, **2009**.

Birnkammer T., Kraus A., Preuss H., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Towards bivalent N^G-acylated hetarylpropylguanidines as potent and selective histamine H_2 receptor agonists", 4th Summer School Medicinal Chemistry, University of Regensburg, September 29 – October 01, **2008**.

"Towards bivalent N^G-acylated hetarylpropylguanidines as potent and selective histamine H_2 receptor agonists", short lecture in occasion of the 4th Summer School Medicinal Chemistry, University of Regensburg, September 29 – October 01, **2008**.

Birnkammer T., Kraus A., Preuss H., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Bivalent acylguanidine-type ligands as potent and selective histamine H_2 receptor agonists", Abstract published in: Drugs of the Future 33 (Suppl. A), 127, 20th International Symposium on Medicinal Chemistry, Vienna, August 31 – September 04, **2008**.

Birnkammer T., Kraus A., Preuss H., Bernhardt G., Dove S., Elz S., Seifert R., Buschauer A., "Bivalent N^G-acylated hetarylpropylguanidines as potent and selective histamine H_2 receptor agonists", Annual Meeting "Frontiers in Medicinal Chemistry", University of Regensburg, March 02 – 05, **2008**.

8.4 Publications and awards

Birnkammer T., Spickenreither A., Brunskole, I., Lopuch M., Bernhardt G., Dove S., Seifert R., Elz S., Buschauer A., The bivalent ligand approach leads to highly potent and selective acylguanidine-type histamine H_2 receptor agonists, *J. Med. Chem.* **2011**.

Ghorai P., Kraus A., Birnkammer T., Geyer R., Bernhardt G., Dove S., Seifert R., Elz S., Buschauer A., Chiral N^G-acylated hetarylpropylguanidine-type histamine H_2 receptor agonists do not show significant stereoselectivity, *Bioorg. Med. Chem. Lett.* **2010**, 20, 3173-3176.

Kraus A., Ghorai P., Birnkammer T., Schnell D., Elz S., Seifert R., Dove S., Bernhardt G., Buschauer A., N^G-Acylated aminothiazolylpropylguanidines as potent and selective histamine H_2 receptor agonists, *ChemMedChem* **2009**, 4, 232-240.

First Prize in the European Histamine Research Society Young Investigator Award: "Synthesis and structure-activity relationships of bivalent acylguanidine-type histamine H_2 receptor agonists", in occasion of the 40th EHRS Meeting, Sochi, Russia, May 11 – 15, **2011**.

"Towards bivalent N^G-acylated hetarylpropylguanidines as potent and selective histamine H_2 receptor agonists", **Poster Award** in occasion of the 4th Summer School Medicinal Chemistry, University of Regensburg, September 29 – October 01, **2008**.

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Regensburg, _____

Tobias Birnkammer