Application of the Guanidine–Acylguanidine Bioisosteric Approach to NPY Y_2 Receptor Antagonists: Bivalent, Radiolabeled and Fluorescent Pharmacological Tools

Dissertation

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Contents

Chapter 1 General Introduction

1.1 Neuropeptide Y

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the central and peripheral nervous system.[1] It was first isolated by Tatemoto *et al.* from porcine brain in 1982.[2] Together with the homologous peptides pancreatic polypeptide (PP) and peptide YY (PYY), NPY belongs to the neuropeptide Y family.[3] For all these peptides, consisting of 36 amino acids, C-terminal amidation is essential for biological activity.[4] The sequence of NPY is highly conserved in various species.[5] The tertiary structure of turkey PP has been elucidated by crystallography.[6-7] In this crystal structure the N-terminal residues 1-8 form a polyproline-like helix, followed by a β-turn (9-13) and an α-helical region (14-31). The C-terminal pentapeptide (32-36), bearing the most crucial residues for receptor recognition, appears rather unordered and flexible. This hairpin-like conformation, the so-called PP-fold, is stabilized by hydrophobic interactions between the polyproline-like N-terminus and the α-helix. Due to high sequence homology of NPY to PP (50 %) this tertiary structure was also proposed for NPY (cf. Figure 1.1).[8]

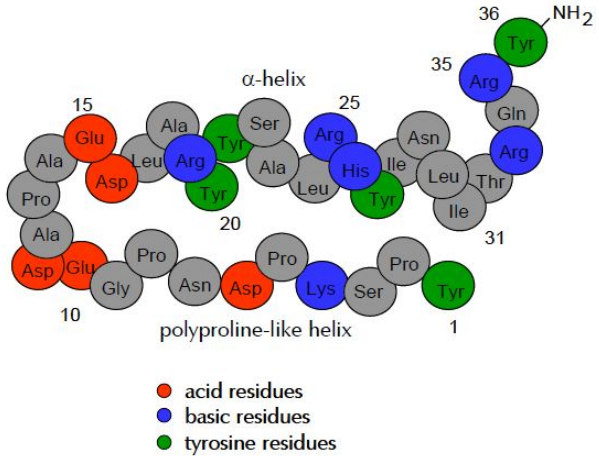

Figure 1.1. Tertiary structure of porcine NPY according to Allen.[8] The residues are arranged according to the crystal structure of the homolog avian PP.

The tertiary structure of NPY in solution was intensively studied by several groups using NMR techniques and CD spectroscopy as well as FRET based approaches. These studies reported contradictory findings, as some confirmed the PP-fold structure[9-11], while others did not find the PP-fold as prevalent conformation of NPY in solution, e.g. due to the formation of dimeric structures through α-helical contacts and conformations with non-helical and flexible N-termini.[12-16] However, conformational studies in solution do not permit any definite conclusions with regard to the receptor-bound conformation due to unphysiologically high concentrations of NPY in the millimolar range. Furthermore, for instance, in NMR studies the pH was adjusted to

values considerably lower than 7.4 in order to increase the solubility of NPY. Considering the results from CD spectroscopic measurements at various concentrations and pH values, different conformations of NPY were observed in a dynamic equilibrium, and the PP-fold conformation is probably favored under physiological conditions.[17] Furthermore, peptide-lipid interactions at the surface of the cell membrane were discussed to support the formation of the active conformation of NPY (cf. Figure 1.2).[18-20]

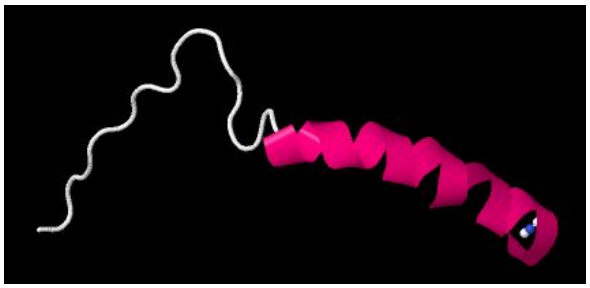

Figure 1.2. The structure of pNPY bound to micelles according to Bader *et al.*[18] The N-terminal part is flexible, while the C-terminal α-helix interacts with the membrane surface. Structural data are obtained from the Protein Data Bank (PDB access code 1F8P, http://www.pdb.org/pdb/home/home.do).

In the peripheral nervous system NPY is expressed in sympathetic neurons, where it is stored together with noradrenaline and functions as cotransmitter.[21] In the central nervous system (CNS) NPY was found in the basal ganglia, hippocampus, amygdala and the hypothalamus. In the CNS, NPY also acts as cotransmitter, e.g., together with noradrenaline, agouti-related peptide (AGRP)[22] or GABA[23].

NPY is primarily synthesized and released by neurons. The biologically active peptide is derived from the 97-amino acid precursor pre-pro-NPY following at least four post-translational enzymatic events (Figure 1.3).[24-27] The 69 residue containing pro-NPY is produced after removal of the signal-peptide (29 amino acids). Then, pro-NPY undergoes cleavage by several enzymes such as prohormone convertase 1 and 2 (PC1+2) and carboxypeptidase E (CPE), thereby generating the mature peptide.

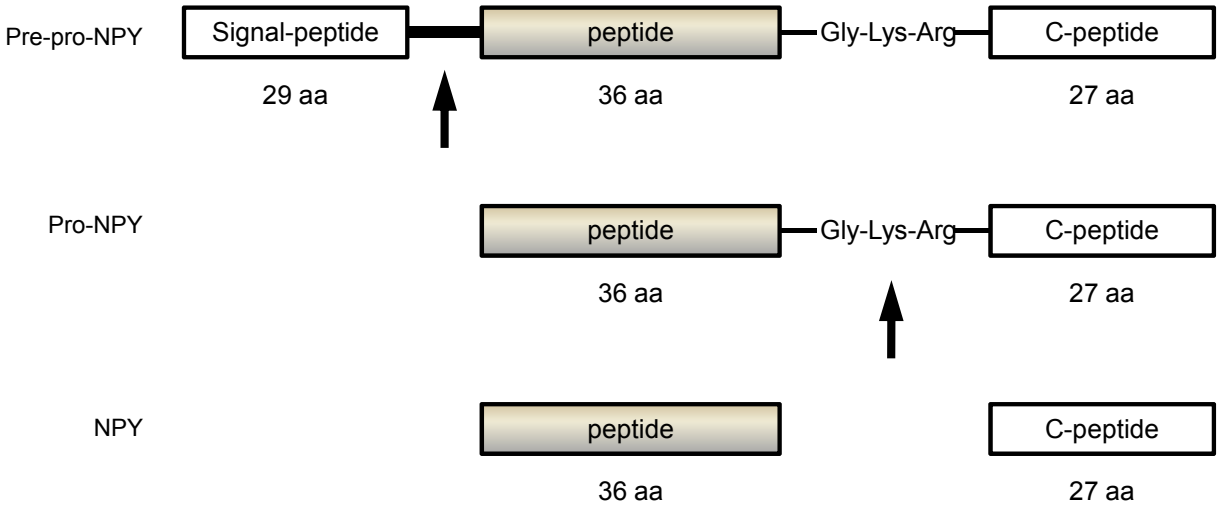

Figure 1.3. Biosynthesis of neuropeptide Y, adapted from von Hörsten *et al.*[28]; aa: amino acid. The arrows schematically present the cleaving sites.

NPY possesses a broad range of biological effects including stimulation of food intake,[29] inhibition of glutamatergic excitatory synaptic transmission,[30] memory processes,[31] hypothermia,[32] anxiolysis,[33] etc. Moreover, NPY is considered to be involved in various pathological processes such as dysregulation of food intake and obesity,[34-37] mood disorders[38-39] and alcoholism.[40]

1.2 Mammalian NPY Receptor Subtypes

The diverse biological effects of NPY are mediated by the activation of different receptor subtypes which are all members of the larger superfamily of G-protein coupled receptors (GPCRs). In mammals five neuropeptide Y receptor subtypes have been described, denoted as Y_1, Y_2, Y_4, Y_5, and y_6.[41-48] The y_6 receptor was found to be functional in mice, but is a non-functional pseudogene in most mammalian species. In the rat genome it is missing at all.[49] In Table 1.1 an overview of the binding profiles, the localization and the physiological role of the mammalian NPY receptor subtypes is given.

Table 1.1. Overview of binding profile, localization and physiological role of the mammalian NPY receptor subtypes.[27, 50-53]

	Binding Profile	Localization	Physiological Role
Y_1	NPY ≈ PYY ≈ [L^{31}, P^{34}]NPY > NPY_{2-36} > NPY_{3-36} ≥ PP > NPY_{13-36}	smooth vascular muscles (postjunctionally), cerebral cortex, hypothalamus, colon, human adipocytes	Involved in regulation of blood pressure, seizures and food intake, anxiety, pain sensitivity, depression, angiogenesis, alcohol consumption
Y_2	NPY ≥ NPY_{2-36} ≈ NPY_{3-36} ≈ NPY_{13-36} >> [L^{31}, P^{34}]NPY	hippocampus, hypothalamus, nerve ends, (pre and postjunctional), renal tubules	Involved in regulation of blood pressure, seizures and food intake, anxiety, pain sensitivity, depression, angiogenesis, hypothalamic regulation of bone formation, regulation of GI motility
Y_4	PP > PYY ≥ NPY > NPY_{2-36}	brain, coronary arteries, ileum	food intake, regulation of GI motility
Y_5	NPY ≈ PYY ≈ NPY_{2-36} > hPP > NPY_{13-36} > rPP	hypothalamus	food intake, seizures, anxiety
y_6	NPY ≈ PYY ≈ [L^{31}, P^{34}]NPY >> PP^{54} **or** PP > [L^{31}, P^{34}]NPY > NPY ≈ PYY^{43}	cardiac and skeletal muscles	no function in humans

1.2.1 The Y_1, Y_4 and Y_5 Receptor

The 384 amino acids containing Y_1R was the first NPY receptor to be cloned in 1992. The functionality of the expressed Y_1R was demonstrated by inhibition of adenylyl cyclase (AC) and mobilization of intracellular calcium. All mammalian Y_1Rs cloned thus far display 90-95 % homology with the human Y_1R (hY_1R).[55] The Y_1R exhibits a high tendency to agonist stimulated internalization, as demonstrated by means of radioligand binding,[56] confocal microscopy[57] and GFP tagged Y_1R.[58] Interestingly, the pseudopeptide Y_1R antagonist GR231118 is able to induce long-lasting disappearance of cell surface Y_1Rs through a mechanism distinct from the classical endocytotic/recycling pathway.[59]

Sequence homology screening using a Y_1R probe led to the isolation of a new human NPY receptor cDNA, encoding for the Y_4 receptor (Y_4R).[47, 60-61] A unique feature of the Y_4R is a high affinity for PP, in contrast to NPY and PYY, which exhibit low affinities for this receptor. Therefore, the Y_4R is also referred to as PP receptor. Sequence homology between human and other species Y_4Rs is one of the lowest (less than 75 %) reported for orthologous GPCRs of different mammalian species.[49] Additionally, the human Y_4R (hY_4R) has higher homology with hY_1R (43 %) than human Y_2 receptor (hY_2R; 34 %).[62] Moreover, there are contrary observations in terms of receptor internalization.[56, 63]

NPY is one of the most potent orexigenic agents and this effect was proposed to be mediated by an atypical Y_1R or "feeding receptor".[64-65] The pharmacological profile of an NPY receptor cloned from human and rat tissues was found to be similar to that of this atypical feeding receptor and classified as the Y_5 receptor subtype (Y_5R).[41, 45] In comparison with other NPY receptor subtypes, this 446 amino acid protein has a very long third intracellular loop with more than 130 amino acids and an unusually short C-terminus. Additionally, sequence homology between the Y_5R and other NPY receptors is very low (≈ 30 %).[49, 55] Lastly, rapid association of β-arrestin 2 with the Y_5R was observed after agonist stimulation, suggesting fast internalization similar to the Y_1R.[66]

1.2.2 The NPY Y_2 Receptor

In 1986, the Y_2 subtype was pharmacologically characterized as a predominantly presynaptic NPY/PYY receptor.[4] The cloned hY_2R consists of 381 amino acids and has only ≈ 30 % identity to the Y_1R and the Y_4R, respectively.[42, 48, 61, 68-69] However, high sequence homology (90-95 %) between species was observed for various Y_2Rs.[49, 55] Activation of the Y_2R leads to an inhibition of AC[70] and in rat neurons to an inhibition of Ca^{2+} influx *via* N-type channels[71-72]. The human glioblastoma cell line LN319 proved useful in defining Y_2-mediated pathways, since these cells exclusively express this NPY receptor subtype.[73] Herein, NPY inhibits forskolin-induced cAMP accumulation and stimulates an increase in intracellular Ca^{2+} *via* pertussis toxin-sensitive pathways.[74] Furthermore, the Y_2R mediates the activation of protein kinase C (PKC) in human ciliated cells.[75] Finally, the Y_2R was also shown to activate MAPK signal transduction through a Gi protein and *via* PI-3 kinase.[76] The different signaling pathways are summarized in Figure 1.4.

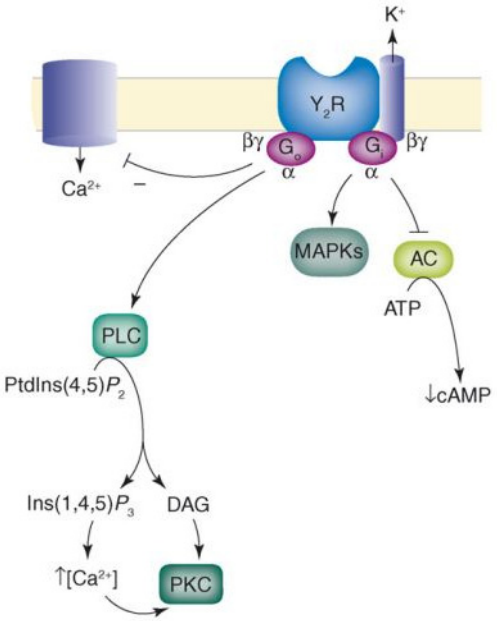

Figure 1.4. The neuropeptide Y Y_2 receptor and its intracellular signaling pathways, adapted from Brumovsky *et al.*[67]

The Y_2R internalization process was a matter of controversy over the past years. Desensitization of the Y_2R after stimulation with NPY was observed in LN319 cells,[74] but unlike the Y_1R, the Y_2R has not been reported to internalize after prolonged agonist excitation[56, 58] or it was reported to internalize very slowly.[73] However, mutagenesis studies recently revealed that substitution of either His155 or His159 by Pro in the intracellular loop 2 (ICL2) can lead to an accelerated internalization.[77] Notably, the postulated regulation of the Y_2R internalization by its ICL2 is inconsistent with the most recent findings on Y_1/Y_2R chimeras, revealing putative inhibitory interactions within the ICL3 and the C-terminal tail of the native Y_2R that reduce internalization.[78] In the meantime, several groups confirmed rapid Y_2R internalization.[79-82]

1.2.3 The Y_2R in Health and Disease

The high sequence homology of Y_2Rs in mammals reflects the involvement of the Y_2R in critical developmental and metabolic events. Thus, the Y_2R is also involved in a variety of human diseases such as epilepsy, obesity and cancer. Especially, the role of the NPY receptor family in appetite regulation has received intense attention in recent years,[83] as obesity has emerged as one of the most serious major human health concerns in the present and future[84]. Herein, the Y_2R appeared as interesting

target as numerous studies reported reduced hunger and food intake in humans after application of the moderately Y_2R selective agonist PYY(3-36).[85-86] The potential benefits of Y_2 and Y_4R agonism for the treatment of obesity resulted in Y_2/Y_4 dual agonists developed by 7TM Pharma. The most promising peptide was tested successfully in Phase I/II trials and is currently under further investigation proving the potential of the Y_2R as anti-obesity drug target.[37] Furthermore, Y_2R agonism was observed to be neuroprotective, presumably, *via* the reduction of glutamate release. Thus, the Y_2R is discussed as a potential drug target in seizures and epilepsy.[87] Moreover, wound healing is reduced in Y_2 knock-out mice.[88] Further functions of the Y_2R which are related to pathological processes were recently summarized.[69]
Besides its physiological implications and potential role in diverse dysregulated physiological processes, the Y_2R attracted strong attendance on its involvement in oncogenesis and has recently been predicted as tumor marker.[89] A remarkably high expression was found in various human brain tumors and mastocarcinoma.[90] Blocking the Y_2Rs led to an inhibition of neuroblastoma growth *in vivo*, emphasizing the possible application of selective and potent Y_2R antagonists in cancer therapy.[91] Recently, a carbaborane modified NPY analog was prepared for potential application in cancer treatment (boron neutron capture therapy).[92] Thus, the Y_2R is considered to be a promising target in tumor therapy and imaging, respectively.

1.3 NPY Receptor Ligands

1.3.1 NPY Y_1, Y_4 and Y_5 Receptor Antagonists

In the last two decades, a multitude of highly potent and selective non-peptidic Y_1R antagonists with affinities in the nanomolar and subnanomolar range have been developed, including BIBP 3226, which is potent and selective except for a moderate affinity for the Neuropeptide FF receptor.[93] A selection of Y_1R antagonists is shown in Figure 1.5.

Recently, an analog of the C-terminus of NPY, the peptide VD-11, was reported to act as a competitive antagonist at the Y_4R.[94] However, high-affinity non-peptide Y_4R ligands are not known so far. The acylguanidines UR-AK49 and UR-PI284 (Figure 1.6) which were designed as histamine H_2 and H_4 receptor ligands, respectively, proved to be weak Y_4R antagonists.[95-96] These compounds may serve as lead structures towards potent small molecule antagonists for the Y_4R.[53]

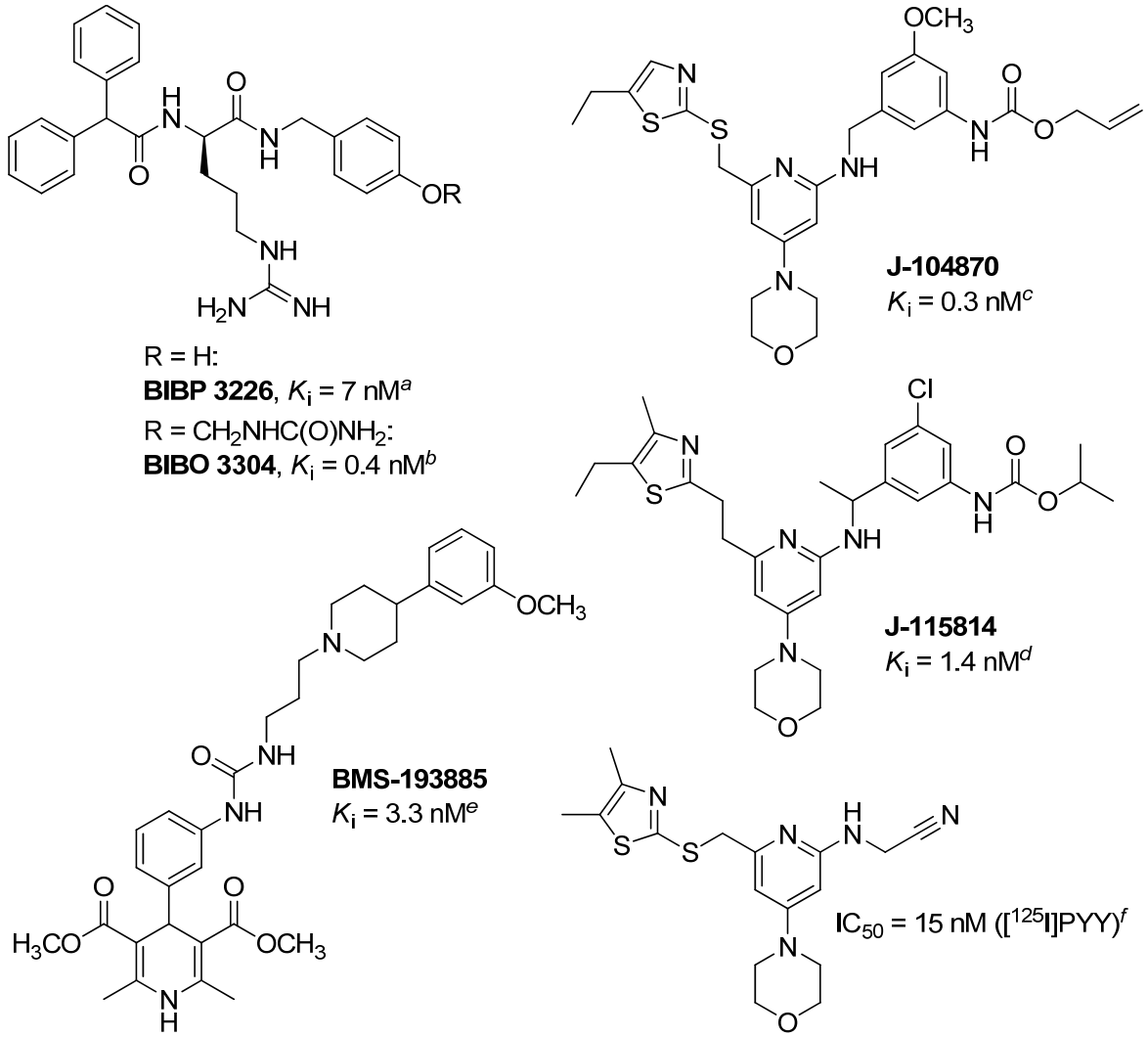

Figure 1.5. Examples of non-peptidic selective Y_1R antagonists. [a] Rudolf *et al.*[97], [b] Wieland *et al.*[98], [c] Kanatani *et al.*[99], [d] Kanatani *et al.*[100], [e] Antal-Zimanyi *et al.*[101], [f] Kameda *et al.*[102]

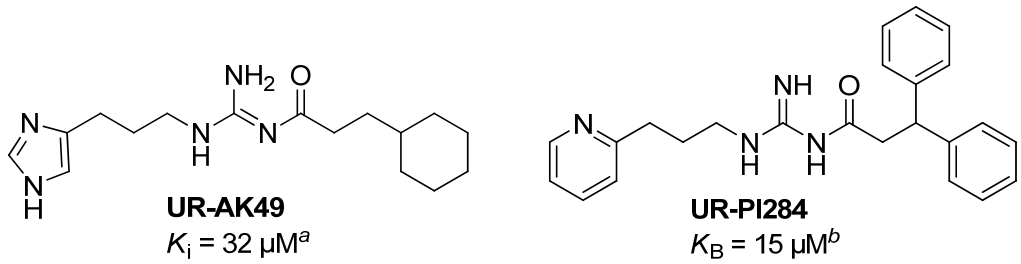

Figure 1.6. Structures of UR-AK49 and UR-PI284, weak Y_4R antagonists; [a] Binding affinity determined in a flow cytometric competition binding assay at CHO-hY_4R-Gα_{qi5}-mtAEQ cells.[103] [b] Antagonistic activity determined in a steady-state GTPase assay at hY_4R containing membranes (hY_4R + Gα_{i2} + G$\beta_1\gamma_2$ + RGS4).[96]

In case of the Y_5R, the situation is similar as for the Y_1R. The search for new anti-obesity drugs led to numerous highly potent and selective non-peptidic antagonists with broad structural diversity. Some of these compounds have entered into clinical trials for the treatment of obesity. MK-0557 (Merck & Co., Inc.) was tested in phase II trials for obesity, but it was withdrawn due to lacking clinically meaningful effects

despite its effects on weight loss.[37] A selection of Y_5R antagonists with affinities in the low nanomolar range is given in Figure 1.7.

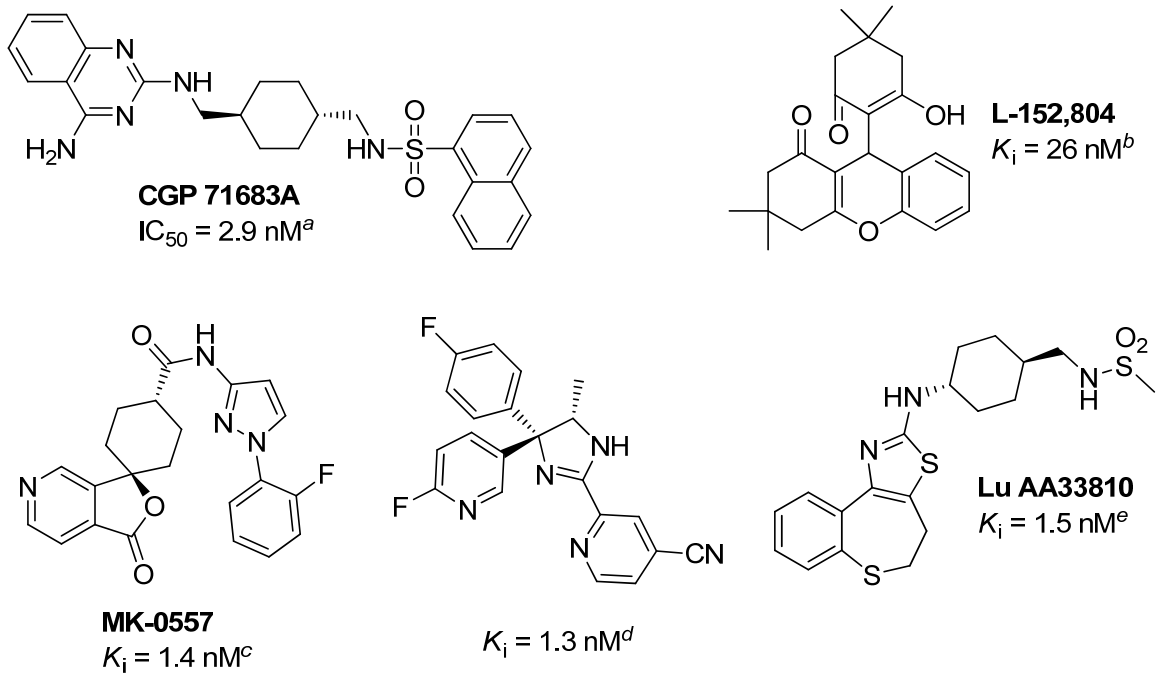

Figure 1.7. Exemplary structures of selective non-peptidic Y_5R antagonists. [a] Criscione *et al.*[104], [b] Kanatani *et al.*[105], [c] Erondu *et al.*[106], [d] Sato *et al.*[107], [e] Walker *et al.*[108].

1.3.2 NPY Y_2R Ligands

Non-peptide small molecule NPY receptor agonists are severely lacking as pharmacological tools and potential drugs. Despite many efforts none have been reported to date. However, several Y_2R selective peptide agonists have been identified in the past. Especially, truncated analogues of NPY and PYY, such as NPY(13-36), PYY(3-36), PYY(22-36) and AcPYY(22-36) exhibited high potency and moderate to high selectivity for the Y_2R.[109-112] Currently, the development of novel truncated PYY analogs, such as modified PYY(22-36) in case of BT-48[113], branched PYY(3-36) derived ligands[114] and substitution of the OH-group of the C-terminal tyrosinamide in PYY(3-36) with a halogen or an amine[115] seems to be the most promising approach for further improvement of the selectivity profile of Y_2R agonists.

First approaches to the design of Y_2R antagonists were also based on truncated NPY analogs.[116] In 1999, the *L*-arginine derivative BIIE 0246 was reported as the first highly selective non-peptide Y_2R antagonist with a one-digit nanomolar affinity.[117] The application of BIIE 0246 for the characterization of the Y_2R revealed a more detailed picture of the receptor´s role in pathological processes. Hence, the disclosed potential of the Y_2R as drug target for several major diseases led to an intensified search for small molecules as Y_2R antagonists in pharmaceutical companies, especially with the help of high throughput screening (HTS). The structures of the

"pioneer" BIIE 0246 and more recent examples for the successful development of small Y_2R antagonists are summarized in Figure 1.8.

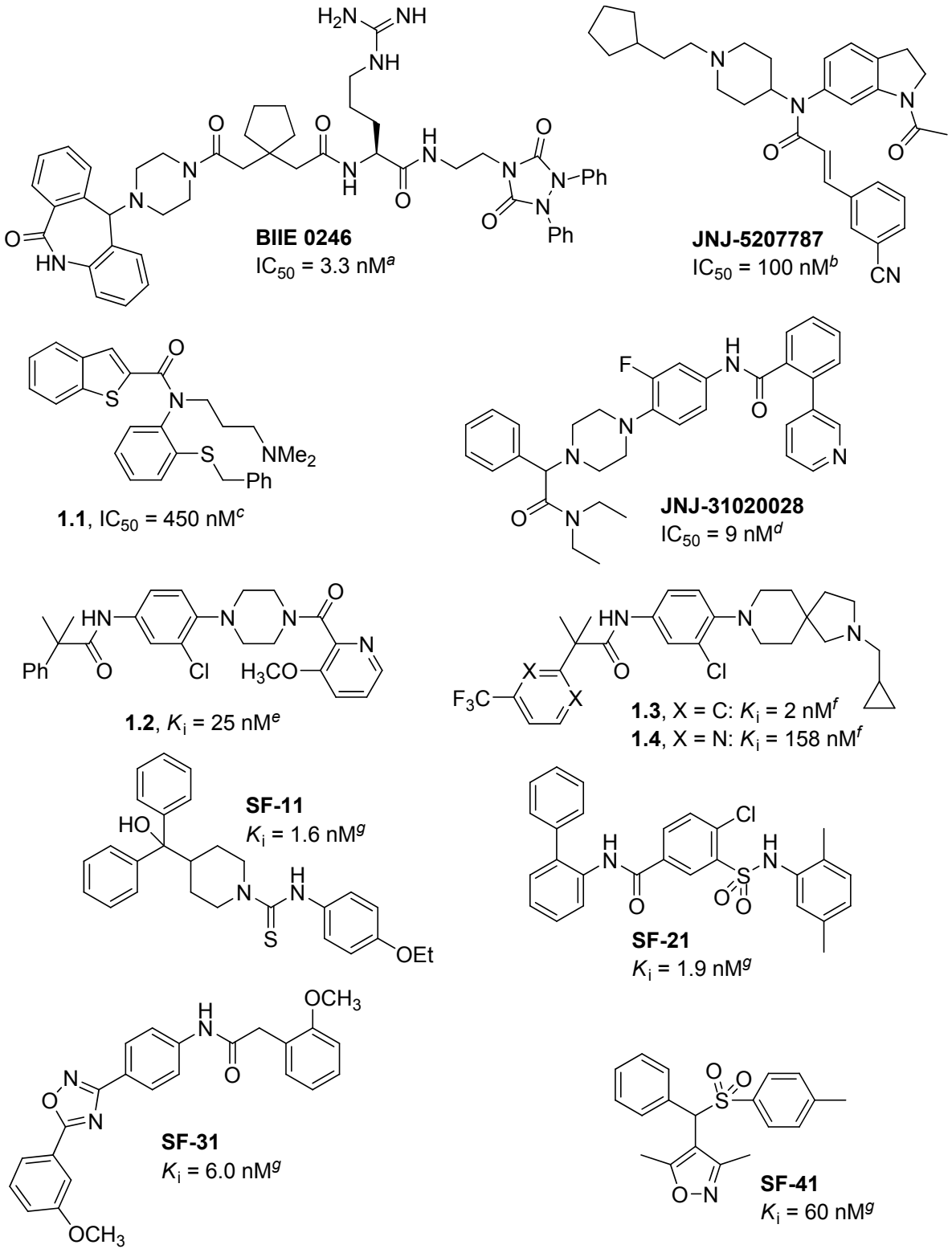

Figure 1.8. Structures of the most potent non-peptide Y_2R antagonists described in literature to date. [a] Doods *et al.*[117], [b] Bonaventure *et al.*[118], [c] Andres *et al.*[119], [d] Shoblock *et al.*[120], [e] Lunniss *et al.*[121], [f] Lunniss *et al.*[122], [g] Brothers *et al.*[123].

The first screening hits with moderate antagonism at the Y_2R were published by Andres *et al*. (Bristol-Myers Squibb; BMS)[119] and Bonaventure *et al*. (Johnson & Johnson; JNJ)[118], respectively. However, it was not before 2009 that small molecules with Y_2R binding affinities in the low nanomolar range were identified. JNJ-31020028, a selective brain penetrant antagonist showed an increased noradrenaline release in the hypothalamus in rats. Yet, the compound was found to be ineffective in a variety of anxiety models although it normalized food intake in stressed animals.[120] An HTS campaign by GlaxoSmithKline (GSK) revealed spiropiperidines **1.3** and **1.4** with improved physicochemical profiles. Compound **1.4** was proposed to be the most promising candidate for further *in vivo* target validation studies despite its lower affinity due to excellent solubility and brain tissue binding.[122] Lastly, Brothers *et al.* described four structurally diverse compounds with nanomolar binding affinities at the Y_2R (SF-11, SF-21, SF-31 and SF-41). All of them are able to cross the blood-brain-barrier and furthermore, selectivity versus 35 GPCRs, two ion channels and three transporters was reported.[123]

1.4 Bioisosteres

The design of bioisosteres introduces certain structural changes that should be beneficial regarding various properties such as size, electronic distribution, polarity, lipophilicity, pK_a, etc. The concept of bioisosterism is versatile in use improving potency, enhancing selectivity, altering physicochemical properties, or altering metabolization and reducing potential toxicity, respectively. The term bioisosterism is subdivided into two broad categories: classical and non-classical. Classical bioisosteres are atoms or functional groups of the same valence (e.g. -F, -OH, $-NH_2$, $-CH_3$) and ring equivalents.[124] Non-classical bioisosteres extend the concept to structural elements that exhibit a more sophisticated form of biochemical mimicry, relying upon functionality. Such modifications can differ quite substantially in electronic, physicochemical and/or steric properties from the functionality being emulated. Hence, a replacement that successfully occurs in a series of compounds addressing a certain target will not necessarily be successful in another class of compounds acting through another receptor.[125-126]

Recent applications of bioisosteres in drug design focus on the newer non-classical approach. For instance, hydroxamic acids are often exchanged in biologically active compounds in order to reduce the toxic potential. The CHF_2 moiety proved to be well suited as a replacement of the hydroxy group in cyclic hydroxamic acids evaluated in a series of dual inhibitors of cycloxygenase-2 and 5-lipoxygenase.[127]

Furthermore, isosteres of carboxylic acid have been studied extensively with the aim to improve membrane permeability and enhance pharmacokinetic properties (for a synopsis see Figure 1.9).[128-131]

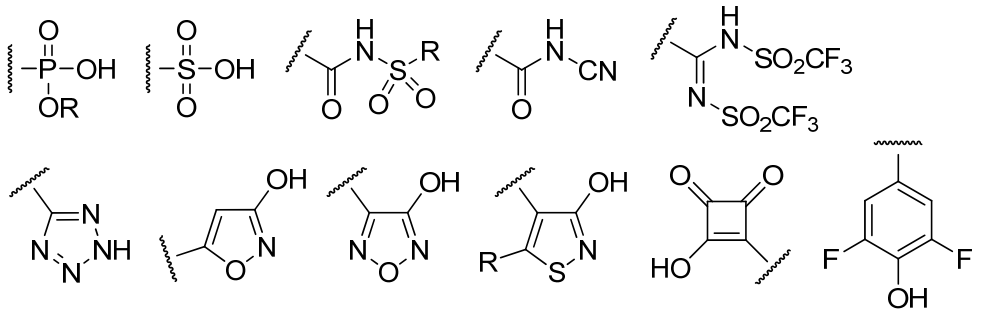

Figure 1.9. Examples of carboxylic acid bioisosteres.

Interest in identifying isosteres of the guanidine and amidine functionality has largely been driven by the disadvantageous physicochemical properties of these moieties, representing highly basic entities. Guanidines (pK_a ≈ 13-14) and amidines are protonated at physiological pH resulting in poor membrane permeability and low oral bioavailability. Basicity can be reduced by 4-5 orders of magnitude (pK_a ≈ 8), affording acylguanidines with improved pharmacokinetic properties. The guanidine-acylguanidine bioisosteric approach was successfully established in our group for different GPCR ligands. Firstly, N^G-acylated imidazolylpropylguanidines were reported to be highly potent histamine H_2 receptor (H_2R) agonists (Figure 1.10).[132-133] The exchange of the imidazole by an aminothiazole ring led to an increased selectivity for the H_2R versus the other histamine receptor subtypes (H_1R, H_3R, H_4R). Thus, two bioisosteric approaches led to improved selectivity profiles and more drug-like properties.[134] Further variations of the N^G-substituent revealed compounds with H_4R selectivity.[135]

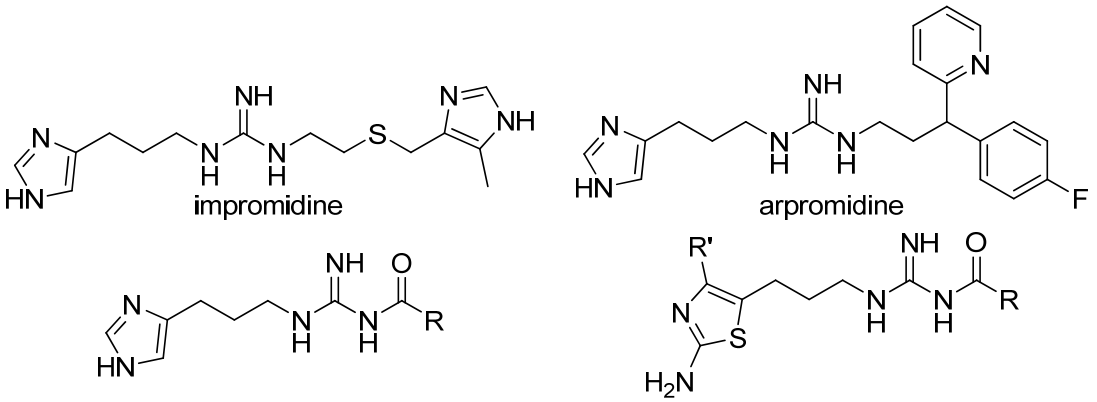

Figure 1.10. Guanidine-type H_2R agonists impromidine and arpromidine and general structures of bioisosteric analogues.

Secondly, acylation of the guanidine moiety of BIBP 3226 using various acyl- or carbamoyl linkers resulted in highly potent and selective Y_1R antagonists with reduced basicity (Figure 1.11). Furthermore, this bioisosteric replacement paved the way for the development of radio- and fluorescence-labeled derivatives.[136-137]

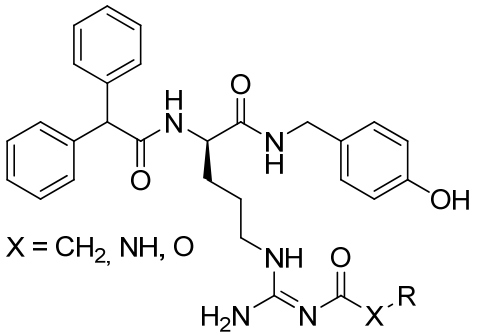

Figure 1.11. General structure of Y_1R antagonists derived from BIBP 3226.

As an alternative to acylation, the basicity of the guanidine moiety can be reduced by the replacement of an adjacent CH_2 with an oxygen atom to yield oxyguanidines (pK_a ≈ 7-7.5), as demonstrated by RWJ-671818, a thrombin inhibitor in phase I clinical studies (Figure 1.12).[138]

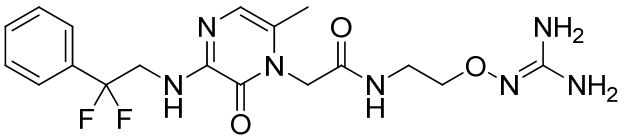

Figure 1.12. Structure of the oxyguanidine RWJ-671818, a thrombin inhibitor.

Lastly, the squaric acid diamide moiety has been successfully explored as a guanidine mimetic of **1.5** in the context of the arginine-derived peptidomimetic **1.6** (Figure 1.13), which inhibits the HIV transcription.[139]

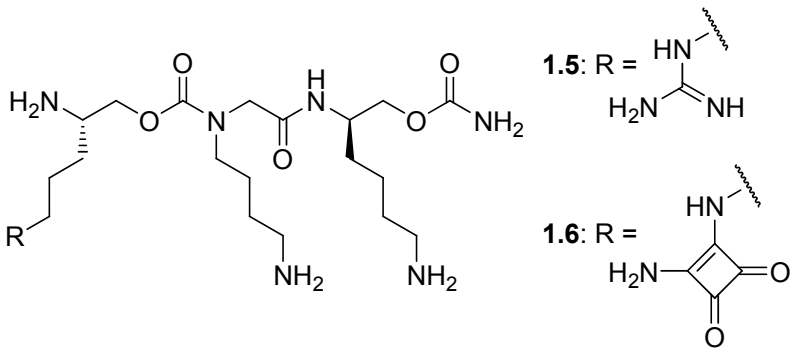

Figure 1.13. Structures of the guanidine **1.5**, an inhibitor of the interaction between the HIV-1 transcription regulator Tat and the Tat-responsive RNA element TAR, and the squaric acid diamide **1.6**, representing a guanidine bioisostere. K_D (**1.5**) = 1.8 μM, K_D (**1.6**) = 7.7 μM.

1.5 Pharmacological Tools

In general, the term "pharmacological tool" defines all types of compounds, which are useful for a detailed pharmacological exploration of a certain target. In case of GPCRs, pharmacological tools are compounds, preferentially antagonists, which bind to a monomeric or oligomeric receptor subtype selectively and with high affinity and provide information about the receptor, for instance, expression, localization, distribution, function, as well as ligand-receptor interactions including binding mode, binding kinetics, etc.

Hence, bivalent antagonists, fluorescence- and radioligands are attractive tools for the characterization of GPCRs and for the investigation of GPCR ligands. In the following, radio- and fluorescence-based techniques commonly applied for the investigation of ligand-receptor interactions with focus on labeled pharmacological tools for NPY receptors will be presented.

1.5.1 Radioligands and Autoradiography

[^{3}H]- and [^{125}I]-labeled ligands are often utilized as standard radioligands in binding experiments and autoradiography, respectively. Tritium is a low energy β^- emitter (max. β energy 18.7 keV). Hence, this radionuclide is often applied in bioassays due to low risk of radiation exposure and simple safety precautions. Radioligand binding experiments are usually performed in order to characterize the binding affinities of novel compounds, as well as for the evaluation of binding properties of the receptor. Binding studies of NPY receptors were performed with variously radiolabeled peptides like [^{3}H]-propionyl-NPY.[140-141] However, selective ligands were needed for the discrimination of receptor subtypes. [^{3}H]BIBP 3226 was reported as a highly potent, reversibly binding Y_1R selective antagonist, useful, for instance, for competition binding assays or the determination of the number of binding sites (B_{max}).[142] Recently, two acylguanidine bioisosteric analogues of BIBP 3226 were reported as tritiated radioligands with decreased basicity and retained Y_1R affinity and selectivity. Besides saturation experiments and kinetics, these ligands were successfully applied to autoradiography.[137, 143]

Receptor autoradiography represents a classical technique for the *in vitro*/*ex vivo* detection of receptor expression and distribution. ^{125}I (γ-emitter, 186 keV) has been applied especially as peptide-label in autoradiographic investigations, for instance, [^{125}I]-NPY and [^{125}I]-PYY as tools for all NPY receptor subtypes except for the Y_4R,[111, 144-147] or [^{125}I]-PYY (3-36) for the detection of the Y_2R[111, 145]. However, there are limitations in the use of ^{125}I, for instance, due to the release of ^{125}I and metabolization/distribution of free iodine in certain tissues (e.g. thyroid gland, stomach, kidneys).[148]

1.5.2 Positron Emission Tomography (PET)

Positron emission tomography (PET) is a powerful molecular imaging technique in medical diagnostics. PET is based on the use of short-lived positron emitting isotopes such as ^{18}F ($t_{1/2}$ = 109.7 min), ^{11}C ($t_{1/2}$ = 20.4 min) or ^{64}Cu ($t_{1/2}$ = 12.7 h). Most commonly ^{18}F is employed as a substitute of a hydrogen atom. The van-der-Waals radii of fluorine and hydrogen are almost the same (1.35 Å vs. 1.20 Å), whereas the differences in electronic properties are very pronounced.

More than 20 nuclear reactions are known for ^{18}F production. Proton bombardment of ^{18}O enriched water resulting in the $^{18}O(p,n)^{18}F$ nuclear reaction is the most effective method and delivers the desired radionuclide with high molar radioactivity.[149] Nowadays, ^{18}F-PET is routinely applied as a diagnostic imaging method in the field of oncology, neurology and cardiology. Most efforts were spent in the development of PET tracers for tumor imaging. For instance, 2-[^{18}F]fluoro-2-deoxy-*D*-glucose ([^{18}F]FDG) is extensively used in diagnosis and therapy control. [^{18}F]FDG, a substrate of glucose transporters, is accumulated in tumor tissues due to metabolic trapping in cancer cells.[150]

As already discussed, NPY receptors are overexpressed in various tumors and therefore, selective PET-ligands are promising pharmacological tools for cancer diagnosis and receptor imaging, respectively. A synopsis of described PET-ligands for the Y_1R and the Y_5R, respectively, is presented in Figure 1.14. For instance, the [^{11}C]-PET ligand **1.7** was prepared as a Y_5R antagonist with high affinity (IC_{50} = 1.5 nM), appropriate lipophilicity (log $D_{7.4}$ = 2.79) and moderate brain penetration (brain/plasma ratio = 0.50). Furthermore, the cold analog proved Y_5R selectivity over the other NPY receptors.[151] The compounds **1.8** and **1.9** were synthesized in our group as prototypical [^{18}F]-PET ligands for the Y_1R, derived from BIBP 3226.[152] 2,4-Diaminopyridine derivatives were identified as promising PET tracer candidates for the Y_1R in terms of binding affinity and lipophilicity by Kameda *et al*.[153] and, recently, one of these compounds was selected for ^{18}F-labeling. The resulting antagonist [^{18}F]Y1-973 does not bind to the Y_2, Y_4 or Y_5 receptor but exhibits Y_1R binding in the subnanomolar range.[154] *In vitro* autoradiography together with *in vivo* PET imaging in rhesus monkey proved the applicability of the novel [^{18}F]-ligand in animal models with potential translation to human PET studies. However, no promising candidates for PET imaging of the Y_2R have been identified yet. Thus, there is urgent need for such compounds to explore the suitability of Y_2R imaging.

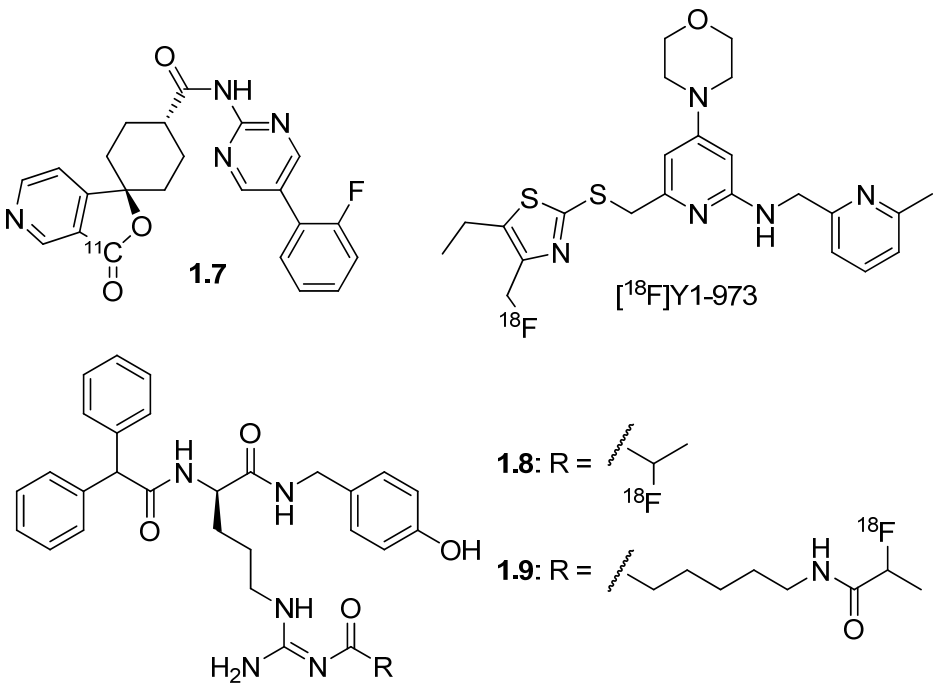

Figure 1.14. PET-ligands for the Y_1R and Y_5R.

1.5.3 Fluorescent Ligand-Based Assays and Fluorescence Imaging

Fluorescence-based binding assays are preferred over radioactive assays in terms of safety precautions and waste disposal. Moreover, numerous fluorescent probes have been developed over the past two decades resulting in the design of potent fluorescence ligands for different GPCRs.[155-162] Thus, there is a wide range of tools, which broaden the scope of application of fluorescence based assays and molecular imaging in GPCR research.

Fluorescence polarization assays are based on the excitation of the sample with polarized light. Free fluorescent ligands emit non-polarized fluorescence after excitation, whereas the emission of receptor bound fluorescent ligands is polarized due to rigidization of the fluorophore in the receptor-ligand-complex. The resulting fluorescence anisotropy allows determination of ligand binding under equilibrium conditions and study of binding kinetics, respectively. As there are no washing steps required in contrast to radioligand binding assays this technique is simply adaptable to HTS.[163]

Various binding assays based on fluorescence resonance energy transfer (FRET) have been developed. Briefly, FRET occurs between a “donor” fluorophore and an “acceptor” fluorophore. As prerequisite for FRET the emission spectrum of the donor must overlap with the excitation spectrum of the acceptor and secondly, the two fluorophores have to be in close proximity to each other (usually < 10 nm). Consequently, this phenomenon was exploited for the detection of GPCR oligomers,

e.g., in case of oxytocine receptors[164] and dopamine D_2/somatostatin sst5 receptor heterooligomers.[165]

1.5.3.1 Flow Cytometry

Flow cytometry provides a sensitive and quantitative method for the measurement of cellular fluorescence. The optical setup of the FACSCalibur™ flow cytometer used in this work is presented in Figure 1.15.

The cytometer is equipped with two lasers, namely an argon laser (488 nm) and a red diode laser (635 nm). Fluorescence resulting from excitation at 488 nm is detected by the photomultipliers Fl-1, Fl-2 and Fl-3, whereas the photomultiplier Fl-4 only detects the red fluorescence emitted after excitation with the red diode laser.

Flow cytometry is widely used for the investigation of numerous cellular and cell-associated parameters, e.g. cell cycle[166], apoptosis[167], oxidant production[168], membrane potential[169], calcium elevation[170] and pH changes[171]. Binding assays for flow cytometric devices have been described e.g. for the chemokine receptor CXCR4[172], the EGF receptor[173] or the α-factor receptor[174]. Recently, flow cytometric equilibrium binding assays for the NPY receptors were established in our group.[95, 175-177] Herein, flow cytometry was successfully applied for the simultaneous determination of binding affinities at the Y_1R, Y_2R and Y_5R, emphasizing the applicability of this technique for HTS.[176]

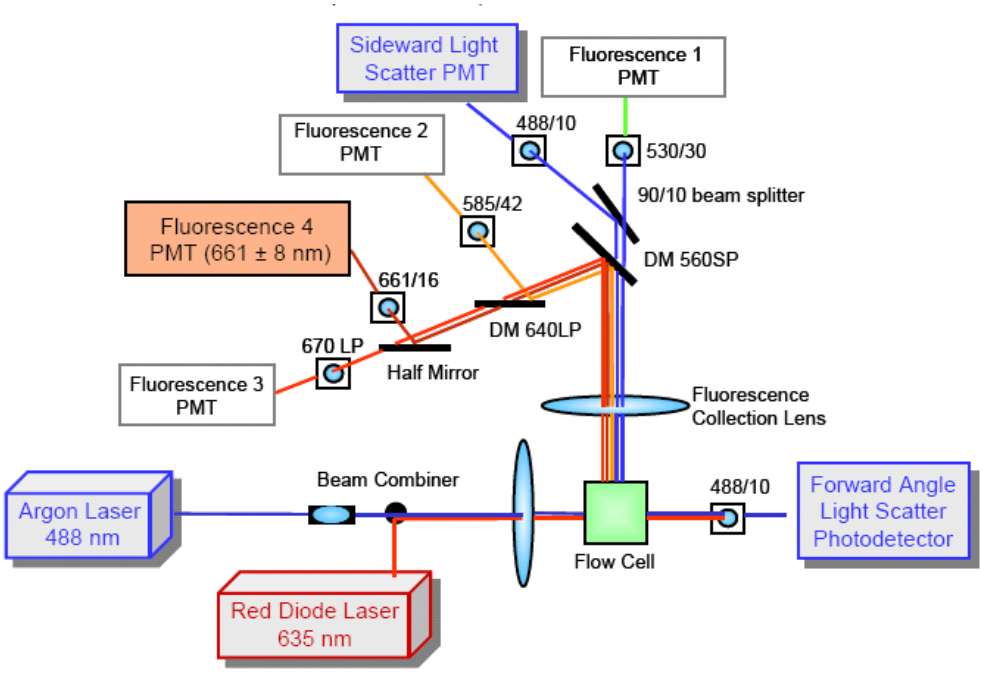

Figure 1.15. Optical setup of the FACSCalibur™ flow cytometer. The fluorescence emission is separated from the SSC light by filters and dichroic mirrors and detected by different photomultiplier tubes (adapted from Mayer[178]).

The main advantage of a flow cytometric binding assay compared to a classic radioligand binding assay is the fact that, similar to fluorescence polarization techniques, the separation of bound and free ligand is not required. Moreover, the sample volume of only several picoliters defined by the intersection of the laser beam

with the sample stream is very small.[179] Therefore, the background signal caused by free fluorophores is very low compared to the signal from the cell. Thus, binding of fluorescent ligands to GPCRs can be determined at equilibrium.

1.5.3.2 Confocal Laser Scanning Microscopy (CLSM)

The number of binding assays using CLSM is rising. For instance, fluorescent adenosine A_1 receptor ligands were characterized by confocal microscopy binding studies at the single cell level.[180] Moreover, quantitative imaging of the native α_1-adrenoceptor with Bodipy-labeled prazosin revealed intracellular high affinity binding sites.[181] Recently, the fluorescence intensity distribution analysis (FIDA) was reported for GPCR-focussed high throughput screening.[182] This technique is applicable to membranes of low GPCR expression levels due to a low detection limit.

Furthermore, confocal microscopy is regarded as an indispensable tool for GPCR imaging. The application of confocal microscopy for the visualization of specifically bound fluorescent peptides, e.g. Bodipy-conjugated NPY ligands or carboxy-fluorescein-NPY for the investigation of NPY receptors, has proven most powerful in studying receptor internalization and trafficking of receptor-ligand complexes into the cells.[57, 183] Lastly, the implication of the Y_1R in the regulation of intracellular Ca^{2+} within the cardiovascular system was also identified by means of confocal microscopy.[184]

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Chapter 2 Scope of the Thesis

Several fluorescent or radiolabeled peptides have been used extensively as pharmacological tools for the investigation of NPY receptor subtypes, since labeled small molecules were not available for routine use in the past. However, such non-peptidic tracers are superior to peptides in terms of their physicochemical properties. Labeled derivatives of the endogenous ligand NPY exhibit limited selectivity and stability. In addition, their applicability as pharmacological tools may be hampered by long incubation periods accompanied with receptor internalization and desensitization due to the slow binding kinetics of peptides. Hence, there is an urgent need for fluorescence and radiolabeled NPY receptor subtype selective low molecular weight antagonists.

The guanidine-acylguanidine bioisosteric approach was extensively studied in our group for the development of pharmacological tools addressing the NPY Y_1 receptor as well as histamine receptor subtypes.[1-4] Inspired by the results from the N^G-acylated Y_1R selective ligands, A. Brennauer prepared the first analogs of the argininamide-type Y_2R selective antagonist BIIE 0246 applying this bioisosterism concept.[5]

The aim of this thesis was the design, synthesis and characterization of non-peptidic tools for the Y_2R. The synthetic strategy is based on the hypothesis that a fluorescent or radioactive label can be attached to the guanidino moiety of BIIE 0246 by an acyl or carbamoyl linker. ω-Aminoacyl linkers were considered most promising, since the fluorophores and radionuclide containing building blocks are commercially available as aminoreactive electrophiles (activated carboxylic acids, pyrylium dyes). Therefore, the present work was focused on the identification and optimization of stable amino-functionalized acyl- and carbamoylguanidines. The fluorescence or radiolabel should be introduced in the final reaction step to yield Y_2R selective tracers. Moreover, the lipophilic pharmacophore of BIIE 0246 should be modified in order to analyze the structure-activity relationships of argininamide-type Y_2R antagonists and additionally to improve the physicochemical properties.

With respect to the synthesis of radioligands for routine use in radioligand binding experiments, tritiated antagonists are preferred in our laboratory, synthesized by acylation with the commonly used tritiated reagent [^{3}H]-propionic acid succinimidyl ester. Such a Y_2R selective radioligand is anticipated to serve as a standard ligand for the determination of binding constants at Y_2R expressing cells and for the detailed investigation of the binding mode of Y_2R selective antagonists.

Beside tritiated compounds, the preparation of potential PET ligands was planned as model compounds towards Y_2R selective [^{18}F]-PET tracers. These ligands are of poten-

tial value as medical diagnostics, since the Y_2R was reported to be expressed in several tumors.[6]

By analogy with this approach, fluorescent Y_2R antagonists differing in the spacer and the fluorophore, respectively, had to be designed, prepared and pharmacologically characterized, since fluorescence labeled compounds offer several advantages compared to radioligands; for instance, in terms of safety precautions, waste disposal or versatile applicability in various fluorescence-based techniques. Thereafter, selected candidates should be investigated in more detail by means of confocal microscopy and flow cytometry. Especially, red-fluorescent Y_2R selective antagonists are regarded promising pharmacological tools for the detection of Y_2 receptors on cells and for the application in equilibrium binding studies. Nevertheless, a major challenge in this field is the preservation of receptor affinity which is usually considerably affected by the introduction of bulky fluorophores into small receptor ligands.

Finally, ligands comprising two pharmacophoric entities are discussed to be of potential value as tools for the investigation of GPCR homodimerization[7-9]. Thus, the feasibility of the bivalent ligand approach was intended to be evaluated by the linkage of two ω-aminoacyl precursors derived from BIIE 0246 with various dicarboxylic acids resulting in bivalent ligands with different chemical nature and length of the spacer.

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Chapter 3 Structure-Activity Relationship Studies of Argininamide-Type Neuropeptide Y Y_2 Receptor Antagonists

3.1 Introduction

In 1999, the non-peptidic (*L*)-arginine derivative BIIE 0246 was disclosed by Boehringer Ingelheim Pharma. This compound proved to be a highly potent and selective neuropeptide Y (NPY) Y_2 receptor (Y_2R) antagonist in binding experiments and functional assays, respectively. Radioligand binding studies on Y_2R expressing SMS-KAN cells revealed an IC_{50} value of 3.3 nM, whereas no displacement of radio-labeled NPY was observed in Y_1, Y_4 and Y_5 receptor assays (Y_1R, Y_4R, Y_5R).[1] Y_2R antagonism was demonstrated, for instance, in pharmacological investigations on the isolated electrically stimulated rat and guinea-pig *vas deferens*.[1-3] More detailed studies on the antagonistic behavior of BIIE 0246 revealed an insurmountable binding mode after pre-incubation of BIIE 0246 with Y_2R expressing cells, whereas competitive antagonism was observed upon simultaneous application of a peptide agonist (e.g. PYY) and BIIE 0246.[4]

BIIE 0246 was among a series of peptidomimetics synthesized as putative mimics of the C-terminus of NPY. For some of these BIIE 0246 analogs Y_2R binding data are given in the patent literature,[5-7] stimulating the discussion of structure-activity relationships of argininamide-type Y_2R antagonists. The 5,11-dihydrodibenzo[*b*,*e*]-azepin-6-one group, though representing the best suited substructure, can be replaced by an α-diphenylmethyl residue resulting in a relatively small decrease in affinity by a factor of 4-5. Apparently, the "western part" of BIIE 0246 undergoes hydrophobic interactions with the receptor. Substitution of the piperazine by a piperidine led to a six times lower affinity.[8] The *L*-arginine side-chain is supposed to form a crucial electrostatic interaction with an acidic residue in the receptor (presumably $Asp294^{6.59}$);[9] exchange by non-basic groups is not tolerated. Interestingly, the replacement of the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione moiety by an (*S*)-tyrosinamide was accepted, whereas the corresponding (*S*)-phenylalaninamide analog showed low affinity. This is in agreement with the assumption that the hydroxyl group is important for receptor binding of BIIE 0246 due to mimicking the C-terminus of NPY.

Mutagenesis studies of the human Y_2R revealed novel insights into the ligand-receptor interactions. Interestingly, BIIE 0246 does not bind to avian Y_2Rs.[10] Based on reciprocal mutations of the human (h) and chicken (ch) Y_2Rs, three amino acids in the

hY_2R were identified to be crucial for BIIE 0246 binding: Gln135$^{3.37}$ in transmembrane domain 3 (TM 3), Leu227$^{5.46}$ in TM 5, and Leu284$^{6.49}$ in TM 6.[11] Point mutagenesis of hY_2R to the corresponding amino acids in chY_2 (Q135H, L227Q, L284F) resulted in low affinity of BIIE 0246. Inversely, the introduction of the three human residues into the chY_2R reproduced the high affinity to hY_2R.[11] A site-directed mutagenesis study revealed the importance of Tyr110$^{2.64}$ in TM 2 of the hY_2R for both, peptide ligands and BIIE 0246 binding.[12] Replacement of this residue by Ala resulted in a 5-9 fold reduction in the affinity of the tested peptide agonists [^{125}I]pPYY, pNPY, hPYY (3-36) and pNPY (13-36). BIIE 0246 displayed a 4-fold reduced affinity for this mutant.[12] The latest site-directed mutagenesis study revealed further amino acids with direct or indirect effects on the interaction of BIIE 0246 with the Y_2R.[13] Therein, L183A exchange resulted in a 46-fold decrease in binding affinity suggesting a direct hydrophobic interaction. Further mutations (T138I, Q287A) implemented a 2.3 and 2.9-fold decrease in affinity, respectively. Interestingly, according to the suggested receptor model, Thr138$^{3.40}$, Leu183$^{4.60}$ and Gln287$^{6.55}$ are oriented towards the cavity defined between TM 3, 4, 5 and 6, which has been previously proposed to accommodate antagonist ligands in the Y_1 receptor.[14-15] These results are first clues towards models of the Y_2R binding site of BIIE 0246.

We are aiming at pharmacological tools for further investigations on the binding mode of BIIE 0246 and related argininamides and for further characterization of the Y_2R, respectively. Several substitutions in the building block of BIIE 0246 were performed in order to improve the physicochemical properties (cf. Figure 3.1). Therefore, the lipophilicity of the building block as well as the basicity of the guanidine should be reduced. Compared to the guanidino group the basicity of acylguanidines is decreased by 4-5 orders of magnitude ($pK_a \approx 8$) but still sufficient to allow for key interactions with acidic residues of the biological target, as demonstrated recently for histamine H_2[16-17] and H_4[18] receptor agonists, Y_1R antagonists[19-21] and a few prototypical Y_2R antagonists[8] from our laboratory. In this work, special attention was paid to further exploring the applicability of the guanidine-acylguanidine bioisosteric approach to Y_2R ligands. Additionally, the synthesis of amino-functionalized building blocks was taken into consideration with respect to the preparation of fluorescent ligands and radiotracers.

The structural features of the synthesized argininamides presented in this chapter are summarized in Figure 3.1. These compounds were investigated at recombinant hY_2Rs for affinity and antagonism, for Y_2R selectivity compared to Y_1R, Y_4R and Y_5R, and for chemical stability. The structure-activity relationships will be discussed.

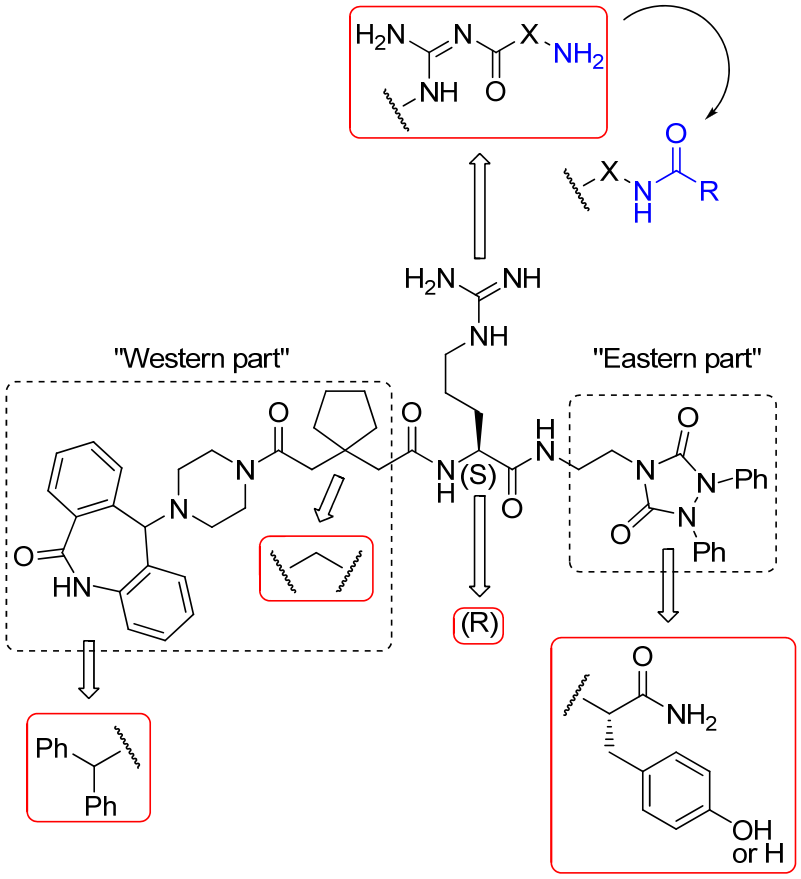
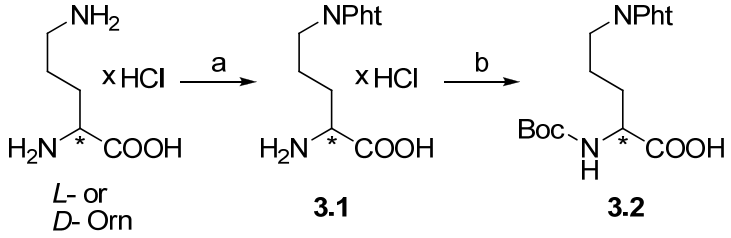


Figure 3.1. Overview about the structural modifications of argininamides described in this chapter.

3.2 Chemistry

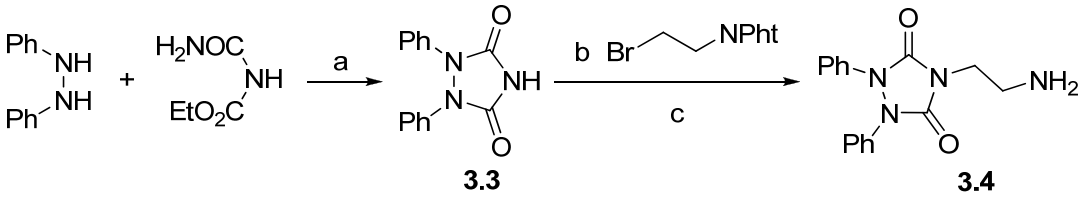
3.2.1 Synthesis

The argininamide-type NPY Y_2R antagonists were synthesized from (*S*)- and (*R*)-configured ornithine (commonly named *L*-Orn and *D*-Orn), respectively. The *tert*-butoxycarbonyl and the phthaloyl group were used as orthogonal protective groups of N^{α} and N^{δ} (Scheme 3.1).



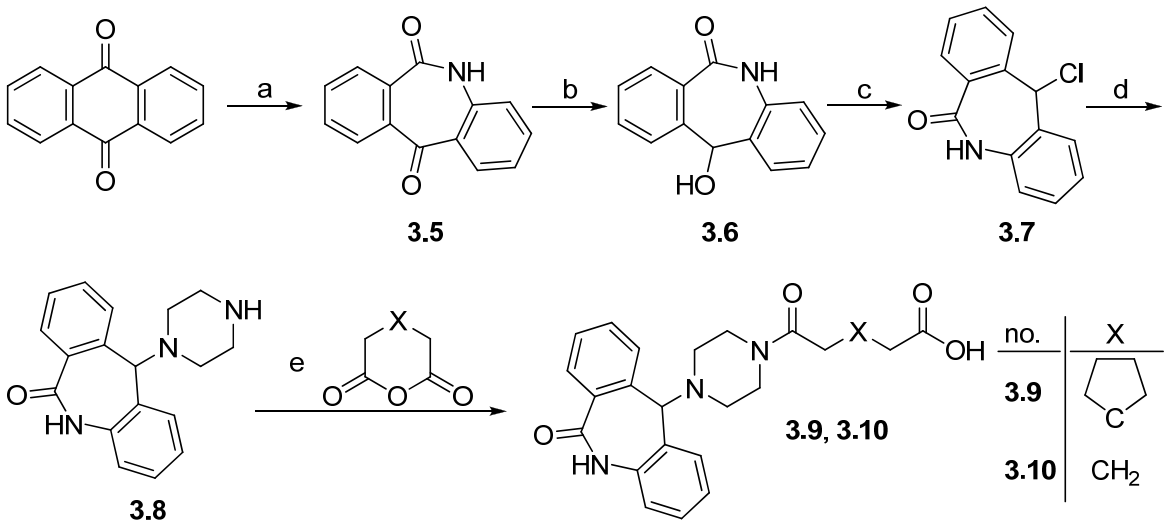
Scheme 3.1. Preparation of orthogonally protected ornithine **3.2**. Reagents and conditions: a) 1. NaOH (2 eq), $CuSO_4 \times 5\ H_2O$ (0.5 eq), $NaHCO_3$ (1.23 eq), H_2O, 30 min, rt, 2. *N*-Carbethoxyphthalimide (1.23 eq), 90 min, rt, 3. 6 M HCl, 90 min, rt; b) Di-*tert*-butyl dicarbonate (1.05 eq), $NaHCO_3$ (2.2 eq), 1,4-dioxane, H_2O, 3 h, rt.

Most of the argininamide-type Y_2R antagonists comprise a 2-(3,5-dioxo-1,2-diphenyl-1,2-triazolidin-4-yl)ethyl group[7] at the C-terminal amide nitrogen. The corresponding amino compound **3.4** was prepared by *N*-alkylation of deprotonated 1,2-diphenylurazol with *N*-(2-bromoethyl)phthalimide, and subsequent hydrazinolysis of the phthaloyl protective group. 1,2-Diphenylurazol was obtained by condensation of 1,2-diphenylhydrazine and ethyl allophanate (cf. Scheme 3.2).[22]



Scheme 3.2. Synthesis of amine component **3.4**. Reagents and conditions: a) xylene, 24 h, reflux; b) KO^tBu (1.10 eq), DMF, 5 h, reflux; c) 1. N_2H_5OH (2.75 eq), THF/MeOH, 16 h, rt, 2. 2 M HCl, 2 h, rt.

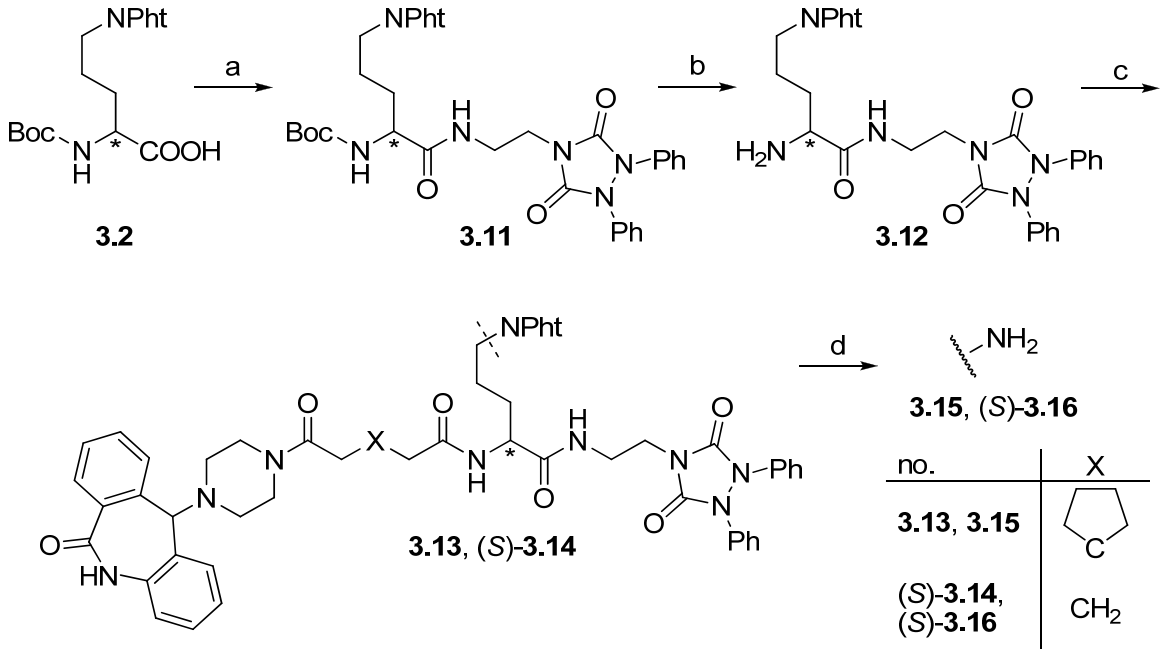
The N^{α} position of the ornithinamide was acylated with either the carboxylic acid **3.9**[7] or **3.10**. For the synthesis of these compounds, anthraquinone was converted into 5*H*-dibenzo[*b*,*e*]azepin-6,11-dione (**3.5**) by Schmidt reaction. The resulting ketone was reduced to alcohol **3.6** by $NaBH_4$ and afterwards converted to the 11-chloro compound (**3.7**) using thionyl chloride. Subsequently, the alkyl chloride was allowed to react with an excess of piperazine yielding 11-piperazin-1-yl-5,11-dihydrodibenzo-[*b*,*e*]azepin-6-one (**3.8**). Finally, acylation of the piperazine with 3,3-tetramethylene glutaric anhydride afforded **3.9**. Carboxylic acid **3.10**, lacking the cycloaliphatic moiety, was prepared by analogy from **3.8** and glutaric anhydride (Scheme 3.3). The synthesis of the ornithinamide building blocks which were subjected to guanidinylation is outlined in Scheme 3.4 and Scheme 3.5.



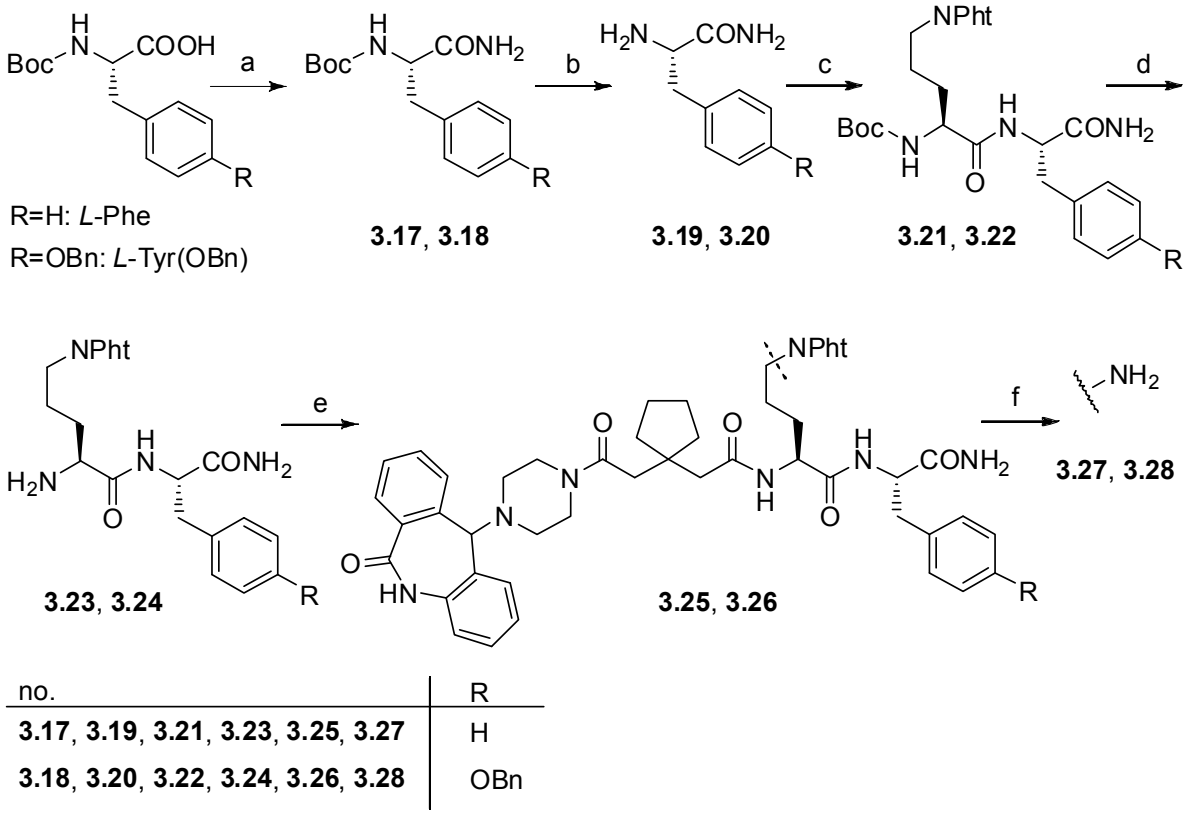
Scheme 3.3. Preparation of the carboxylic acids **3.9**, **3.10**. Reagents and conditions: a) NaN_3 (1.2 eq), H_2SO_4, $CHCl_3$, 10 h, reflux; b) $NaBH_4$ (2 eq), EtOH, 1 h, reflux; c) $SOCl_2$, $CHCl_3$, 30 min, reflux; d) piperazine (5 eq), 1,4-dioxane, 30 min, reflux; e) anhydride (1 eq), CH_2Cl_2, 18 h, rt.

For the preparation of the phthaloyl-protected ornithine amide building blocks **3.13** and **3.14**, the N^{α},N^{δ}-diprotected ornithine **3.2** reacted with the amine **3.4** after *in situ* activation with DCC, followed by Boc-deprotection and a second EDC/HOBt promoted coupling step with the carboxylic acids **3.9** or **3.10** (Scheme 3.4) yielding **3.13** and **3.14**, respectively.[8] The phthaloyl group was cleaved off by hydrazinolysis, giving the primary amines **3.15** and **3.16**.

The BIIE 0246 analogs comprising a phenylalanine amide or a tyrosine amide in the "eastern part" instead of the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione moiety were prepared starting from the (*S*)-configured *tert*-butoxycarbonyl protected phenylalanine, and the *N*-Boc,*O*-Bn protected (*S*)-tyrosine, respectively (Scheme 3.5). The amino acids were converted into the respective amides (**3.17**, **3.18**) by ammonolysis. Therefore, the carboxylic acids were activated with isobutyl chloroformate (IBCF) followed by amidation with 7 M NH_3 in methanol.[23-24] Deprotection, acylation with the orthogonally protected ornithine, and a second acidic Boc-group elimination resulted in the dipeptides **3.23** and **3.24**. Subsequent acylation with **3.9** gave the Pht-protected building blocks **3.25** and **3.26**. The free amines **3.27** and **3.28** were obtained after hydrazinolysis of the N^{δ}-Pht protected compounds (Scheme 3.5).



Scheme 3.4. Preparation of the ornithinamide building blocks **3.15**, (*S*)-**3.16**. Reagents and conditions: a) **3.4** (1.15 eq), HOBt × H_2O (1 eq), DCC (1 eq), THF, 18 h, 0 °C → rt; b) HCl in ethyl acetate, 2 h, rt; c) **3.9** or **3.10** (1 eq), HOBt × H_2O (1 eq), EDC × HCl (1 eq), DIPEA (2 eq), CH_3CN, 16 h, 0 °C → rt; d) 1. N_2H_5OH (2.5 eq), MeOH, 16 h, rt, 2. 1 M $KHSO_4$ (5 eq), 3 h, rt.

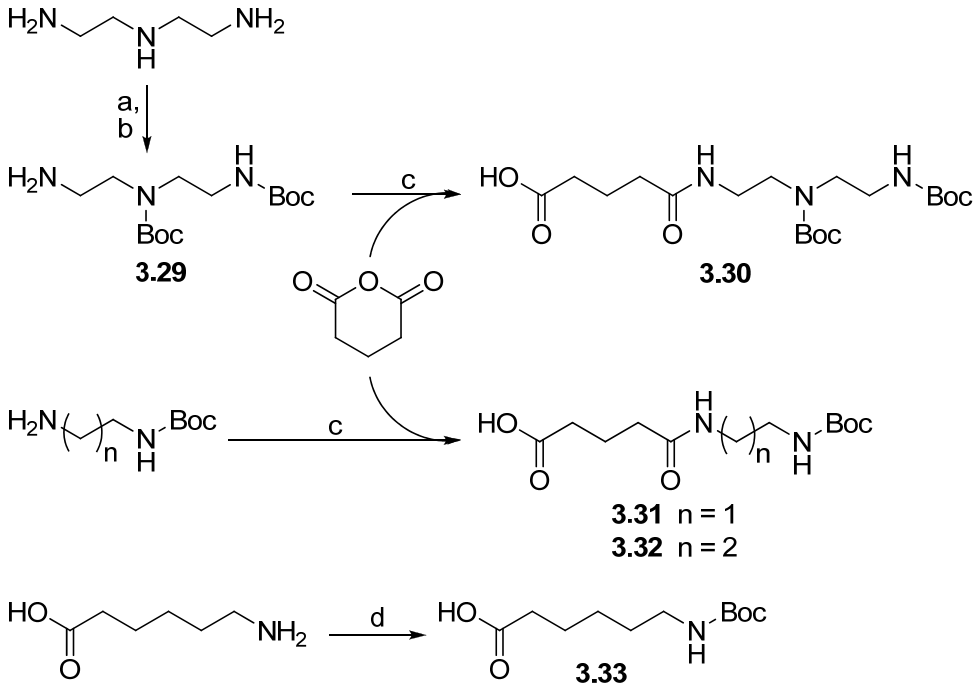


Scheme 3.5. Preparation of building blocks **3.27**, **3.28**. Reagents and conditions: a) 1. IBCF (0.7 eq), NEt_3 (0.7 eq), THF, 30 min, 0 °C → –78 °C, 2. 7 M NH_3 in MeOH, 2 h, –78 °C → rt; b) TFA/CH_2Cl_2 1:1 (v/v), 2 h, rt; c) (*S*)-**3.2** (1 eq), TBTU (1 eq), DIPEA (2 eq), DMF, 16 h, rt; d) HCl in ethylacetate, 2 h, rt; e) **3.9** (1 eq), TBTU (1 eq), DIPEA (2 eq), DMF, 16 h, rt; f) 1. N_2H_5OH (2.5 eq), MeOH, 16 h, rt, 2. 1 M $KHSO_4$ (5 eq), 3 h, rt.

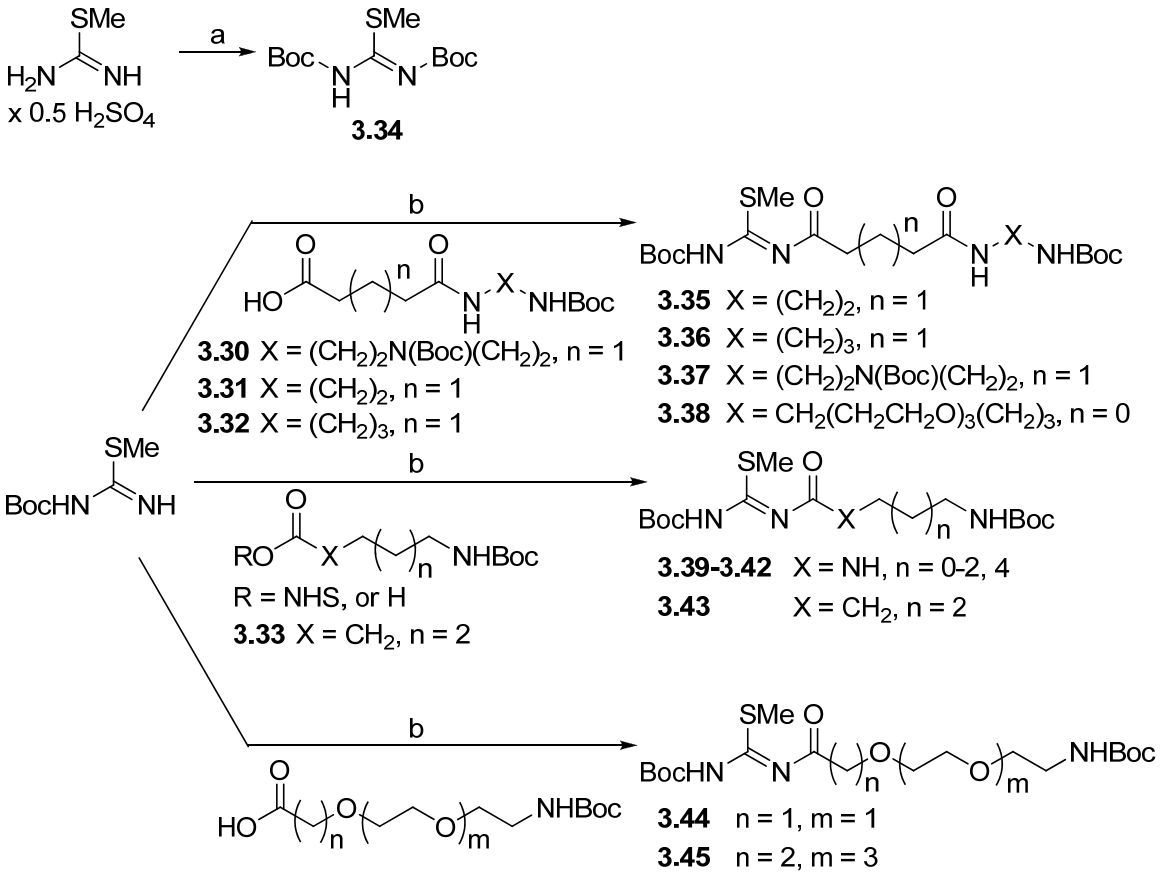
For the preparation of the acylguanidines by guanidinylation of ornithine derivatives, variously substituted *S*-methylisothioureas were synthesized as building blocks (**3.35**-**3.45**; Scheme 3.7). Therefore, mono Boc-protected *S*-methylisothiourea was acylated with the corresponding carboxylic acid either by *in situ* activation with TBTU or by coupling with the pertinent succinimidyl ester. The carboxylic acids **3.30**-**3.33** which were not commercially available were prepared as outlined in Scheme 3.6. In case of the N^G-unsubstituted parent compounds BIIE 0246 ((*S*)-**3.47**), (*R*)-**3.47**, **3.46**, (*S*)-**3.59**, and **3.61**, the guanidine moiety was introduced by treating the primary amine with *N*,*N*'-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **3.34**.

Finally, the N^G-unsubstituted argininamides, as well as the acylguanidine analogs (**3.47**-**3.62**) were prepared by guanidinylation of the corresponding building block with the appropriate *S*-methylisothiourea and subsequent Boc-deprotection with 50 % TFA in dichloromethane (Scheme 3.9).[16, 20, 25]

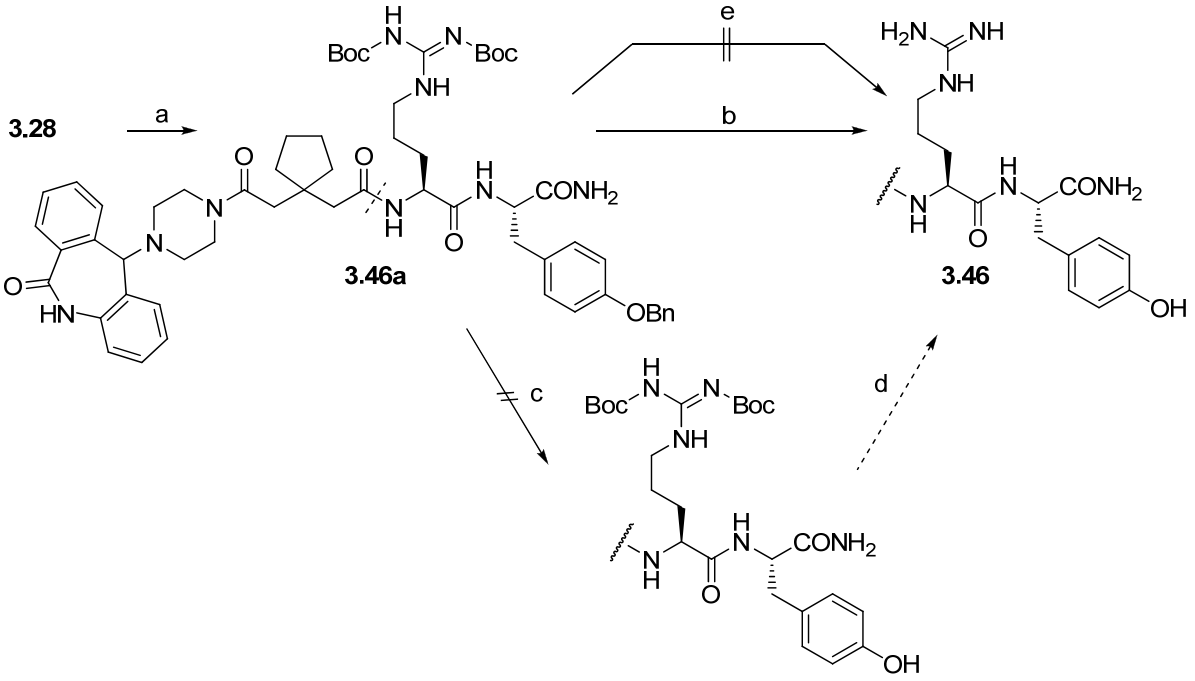
For compound **3.46a**, comprising an *O*-benzyl protected tyrosine, a different cleavage strategy had to be elaborated (Scheme 3.8). A two step reaction starting with hydrogenolysis of the benzyl ether followed by acidic Boc deprotection failed due to insufficient stability of the compound under reductive conditions. Therefore, different non-reductive procedures were tested. Lastly, a one step cleavage of both protective groups was achieved by the treatment with a 1 M solution of trimethylsilyl triflate and thioanisole 1:1 (n/n) in TFA and m-cresole, whereby thioanisole and m-cresole act as cation scavengers, to yield the tyrosinamide analog **3.46**.[26]



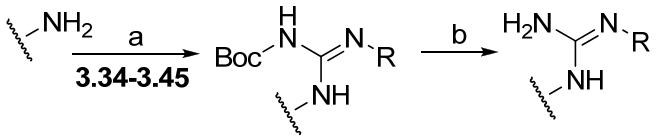
Scheme 3.6. Synthesis of the carboxylic acids **3.30**-**3.33**. Reagents and conditions: a) Ethyl trifluoroacetate (1 eq), CH_2Cl_2, 4 h, 0 °C → rt; b) 1. Di-*tert*-butyl dicarbonate (2 eq), CH_2Cl_2, 24 h, –10 °C → rt, 2. K_2CO_3 (10 eq), CH_3OH, H_2O, 2 h, reflux; c) for **3.30**: NEt_3 (1.5 eq), THF, overnight, rt; for **3.31** and **3.32**: CH_2Cl_2, 2 h, rt; d) Di-*tert*-butyl dicarbonate (1.2 eq), $NaHCO_3$ (1.5 eq), 1,4-dioxane, H_2O, 16 h, rt.



Scheme 3.7. Synthesis of the guanidinylating reagents **3.34**-**3.45**. Reagents and conditions: a) Di-*tert*-butyl dicarbonate (5 eq), 1 M NaOH, H_2O/1,4-dioxane 1:1 (v/v), overnight, rt; b) for R = H (**3.33**): TBTU (1 eq), DIPEA (2 eq), DMF, 16 h, rt; for R = NHS: DIPEA (2 eq), DMF, 16 h, rt.



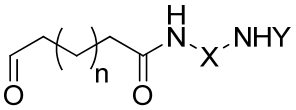
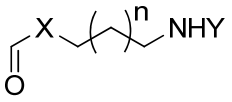
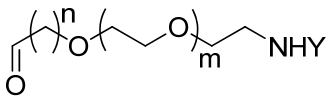
Scheme 3.8. Different deprotection strategies for the synthesis of compound **3.46**. Reagents and conditions: a) **3.34** (1 eq), NEt_3 (2 eq), $HgCl_2$ (1 eq), DMF, 16 h, rt; b) TMSOTf (90 eq), $PhSCH_3$ (90 eq), m-cresole (10 eq), TFA, 90 min, 0 °C; c) H_2, 10 % Pd/C, MeOH, 3 h-overnight, rt; d) TFA/CH_2Cl_2 1:1 (v/v); 2 h, rt; e) TMSI (5 eq), CH_2Cl_2, 4 h, reflux.



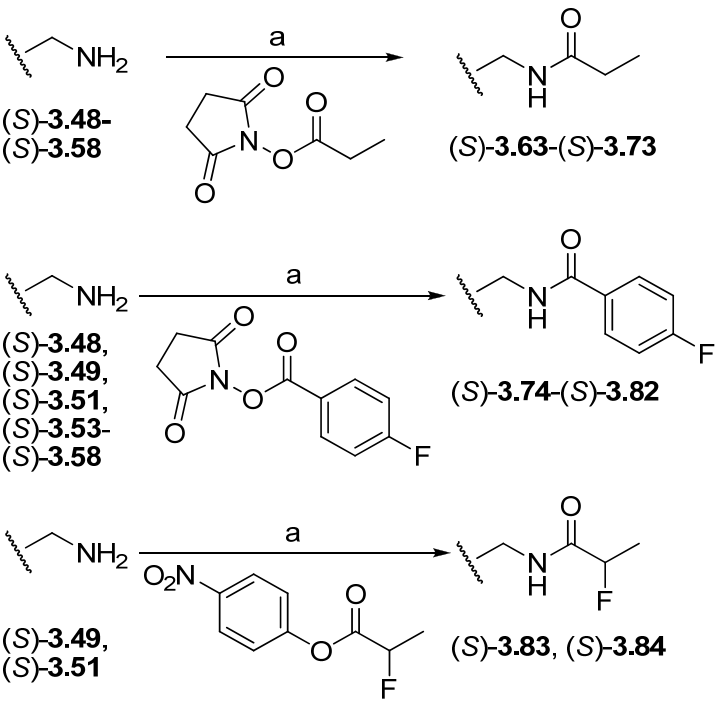
3.15	**3.47a-3.58a**	**3.47-3.58**
3.16	**3.59a-3.60a**	**3.59-3.60**
3.27	**3.61a-3.62a**	**3.61-3.62**

3.47a, **3.59a**, **3.61a**: R = Boc
3.47, **3.59**, **3.61**: R = H

no.	Y
3.48a-3.58a, **3.60a**, **3.62a**	Boc
3.48-3.58, **3.60**, **3.62**	H

R =		
	**3.48a**, **3.48**	X = $(CH_2)_2$, n = 1
	3.49a, **3.49**	X = $(CH_2)_3$, n = 1
	3.50a, **3.50**, **3.60a**, **3.60**	X = $(CH_2)_2NY(CH_2)_2$, n = 1
	3.51a, **3.51**	X = $CH_2(CH_2CH_2O)_3(CH_2)_3$, n = 0
	**3.52a-3.55a**, **3.52-3.55**	X = NH, n = 0-2, 4
	3.56a, **3.56**	X = CH_2, n = 2
	3.62a, **3.62**	X = NH, n = 1
	**3.57a**, **3.57**	n = 1, m = 1
	3.58a, **3.58**	n = 2, m = 3

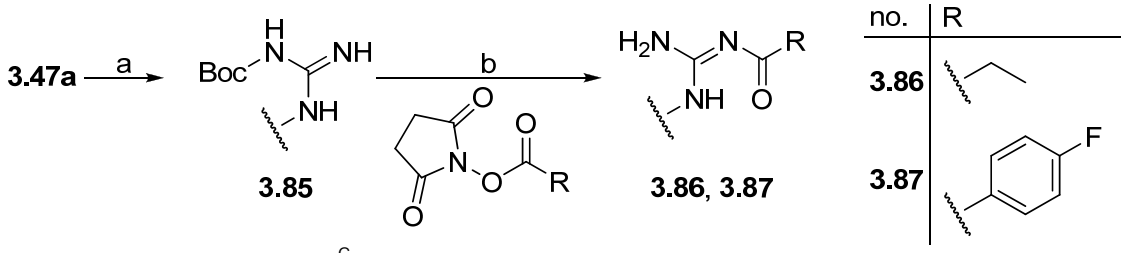
Scheme 3.9. Preparation of N^G-unsubstituted Y_2R antagonists **3.47**, (*S*)-**3.59**, **3.61** and N^G-(ω-aminoacylated) argininamides **3.48**-**3.58**, (*S*)-**3.60**, **3.62**. Reagents and conditions: a) NEt_3 (2 eq), $HgCl_2$ (1 eq), DMF, 16 h, rt; b) TFA/CH_2Cl_2 1:1 (v/v), 2 h, rt.



Scheme 3.10. Preparation of the potential radio- and PET-ligands (*S*)-**3.63**-(*S*)-**3.84**. Reagents and conditions: a) NEt_3 (2.5 eq), CH_3CN, 4-16 h, rt.

Finally, N^G-acylated or carbamoylated guanidines containing a terminal amino group were acylated using the corresponding active esters of propionic acid, 2-fluoro propionic acid, or 4-fluoro benzoic acid in order to obtain a small library of the "cold form" of potential [^{3}H]-radio- and [^{18}F]-PET-ligands for the Y_2R (Scheme 3.10).[21] In addition, compounds **3.86** and **3.87** which are bearing the potential radiolabeling group at the guanidine moiety were prepared (Scheme 3.11). Due to the high basicity of the guanidine moiety a direct acylation of the N^G-unsubstituted Y_2R antagonist **3.47** was hardly possible. Hence, an alternative synthetic route was developed. Commonly, in our lab the guanidinylating reagent is introduced as a Cbz/Boc protected building block followed by reductive cleavage of the Cbz group, acylation and final Boc cleavage.[21, 27] However, the ornithinamide core structure of all Y_2R antagonists turned out to be unstable under reductive hydrogenolysis conditions. Thus, the commonly used orthogonal protective group strategy could not be applied. In principle, the guanidinylation of primary amino groups using the pertinent *S*-methylisothioureas containing propionyl, 2-fluoropropionyl or 4-fluorobenzoyl residues is synthetically feasible. However, the application of this approach is restricted to non-radioactive compounds. The introduction of the radiolabel into the guanidinylating agent would require additional reaction steps with radioactive material and therefore complicate the synthesis in terms of waste disposal, safety precautions and reaction time. Such a synthetic strategy is especially disadvantageous in case of [^{18}F]-labeled ligands due to the very short half-life of the radioisotope ^{18}F ($t_{1/2}$ = 109.7 min). Therefore, introduction of the radiolabel in the final reaction step is preferred.

The mono-Boc protected argininamide **3.85** was synthesized by guanidinylation with the bis-Boc protected *S*-methylisothiourea **3.34** followed by acidic cleavage of only one *tert*-butoxycarbonyl substituent. The deprotection under acidic conditions using mixtures of CH_3CN and water containing 0.1 % TFA was monitored by HPLC and was found to be quantitative after 3 h (Figure 3.2). The N^G-propionylated, and 4-fluoro-benzoylated analogs (**3.86**, **3.87**) were obtained after acylation with the corresponding NHS-ester and a second Boc-deprotection in one pot (Scheme 3.11).



Scheme 3.11. Preparation of N^G-acylated analogs **3.86**, **3.87**. Reagents and conditions: a) CH_3CN/0.1 % aq. TFA 1:1 (v/v), 5 h, rt; b) 1. NHS-ester (1.2 eq), NEt_3 (1 eq), CH_3CN, 21 h, rt, 2. TFA, 2 h, rt.

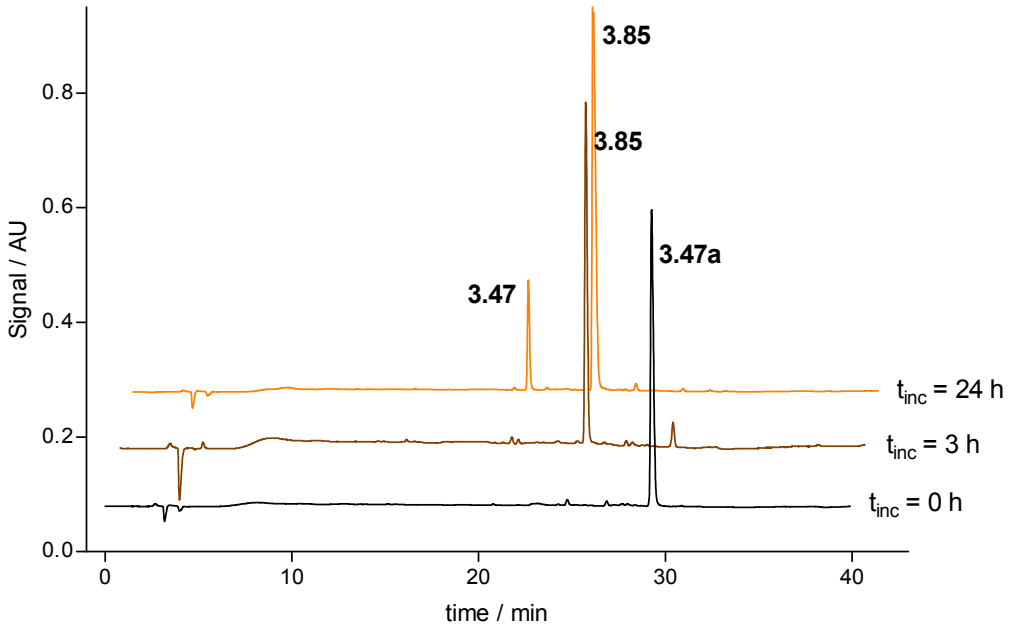


Figure 3.2. HPLC reaction monitoring of the selective cleavage of one *tert*-butoxycarbonyl protective group in **3.47a** resulting in compound **3.85**. Conditions: eluent: mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5, 30 to 40 min: 95/5, t_R (**3.85**) = 24.7 min.

3.2.2 Stability of the NPY Y_2R Antagonists

As recently reported, N^{ω}-acylated argininamides have to be considered critical with respect to the stability, depending on the structure of the acyl substituent.[25, 28] Therefore, a selection of compounds, which were considered promising candidates for fluorescence-labeling and the synthesis of radioligands, respectively, were investigated with respect to decomposition giving BIIE 0246 ((*S*)-**3.47**) under assay-like conditions (aqueous buffer, pH 7.4, 20 °C, time scale 90 min, cf. Table 3.1). A release of (*S*)-**3.47** under the conditions of the pharmacological assays has to be taken into account, as the high potency of *in situ* generated (*S*)-**3.47** (K_i = 10.2 nM) might mask low activity of the (decomposing) Y_2R antagonist. In case of the propionylated potential radioligands and most of the amine precursors only traces of the parent compound BIIE 0246 were detected after 90 min, whereas the decomposition of the amine (*S*)-**3.48** and the fluorobenzoyl derivative (*S*)-**3.74** was much more pronounced. Interestingly, propionylation of the amine group in the linker of (*S*)-**3.48** yielding (*S*)-**3.63** stabilized the compound to a high degree (Figure 3.3a, b). Cleavage of aminoalkanoylguanidines was already observed for N^{G}-acylated argininamide-type Y_1R antagonists and was proven to be extremely rapid in case of structures favoring an intramolecular nucleophilic attack at the carbonyl group, for instance, (5-aminopentanoyl)guanidines.[25, 28] Nevertheless, the affinity of the majority of compounds examined in this study was not affected by the formation of BIIE 0246, as the extent of decomposition was very low. Especially noteworthy is the

high stability of the carbamoylated analogs (Figure 3.3c, d), which was also demonstrated in recent studies on fluorescent Y_1R antagonists.[20]

Table 3.1. Decomposition of selected Y_2R antagonists in buffer (pH 7.4) at 20 °C during incubation for a period of 90 min.

No	% Decomposition	No	% Decomposition	No	% Decomposition
(*S*)-**3.48**	11.6	(*S*)-**3.49**	<0.5	(*S*)-**3.50**	2.9
(*S*)-**3.53**	<0.5	(*S*)-**3.54**	<0.5	(*S*)-**3.55**	<0.5
(*S*)-**3.57**	2.4	(*S*)-**3.58**	<0.5	(*S*)-**3.63**	2.7
(*S*)-**3.69**	<0.5	(*S*)-**3.72**	2.4	(*S*)-**3.74**	5.9

Cleavage of the acyl or carbamoyl substituent from the guanidine group resulting in BIIE 0246 ((*S*)-**3.47**) was analyzed by HPLC.

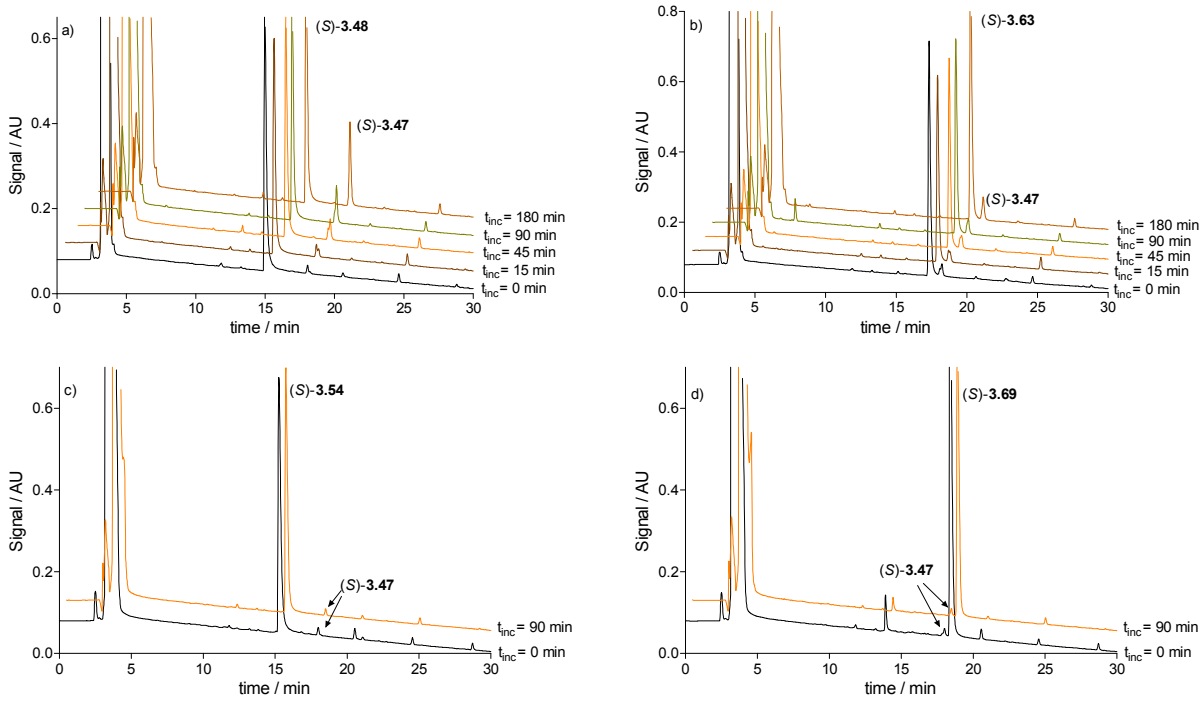


Figure 3.3. HPLC analysis of the chemical stability of N^{ω}-substituted Y_2R antagonists. (*S*)-**3.47** is formed by decomposition of the ligands. Incubation: 15-180 min in buffer, pH 7.4, 20 °C. a) and b) illustrate the chemical stability of the N^{ω}-aminoacylguanidine (*S*)-**3.48** and the corresponding propionylated analog (*S*)-**3.63**. c) and d) show the HPLC analysis of the N^{ω}-aminocarbamoylguanidine (*S*)-**3.54** and the corresponding "cold" radioligand (*S*)-**3.69**.

3.3 Pharmacological Results and Discussion

The Y_2R antagonists were investigated for Y_2R binding and antagonism as well as for Y_2R selectivity over Y_1, Y_4 and Y_5 receptor (Y_1R, Y_4R, Y_5R). The Y_2R binding affinities were determined in a flow cytometric binding assay using CHO cells, stably expressing the human Y_2 receptor (hY_2R),[29] and fluorescence-labeled pNPY (Cy5-pNPY or Dy-635-pNPY). NPY Y_2R antagonistic activities were determined in a spectrofluorimetric Ca^{2+} assay (fura-2 assay) on CHO cells, stably expressing the hY_2R.[30] Cell clones giving a robust intracellular Ca^{2+} response upon agonistic activation were obtained by co-transfection of the CHO-hY_2 cells with the gene encoding the chimeric G-protein Gq_{i5}. Recently, an aequorin-based bioluminescence assay for the Y_2R was developed in our laboratory.[29] Thus, the CHO-hY_2 cells were additionally transfected with the mtAEQ construct, which did not affect the functional properties of the hY_2R (CHO-hY_2-K9-qi5-K9-mtAEQ-A7).

SAR Studies of Argininamide-Type NPY Y_2 Receptor Antagonists: Y_2R Antagonism, Binding Affinity and Physicochemical Properties

The structures and pharmacological data of the investigated argininamide-type Y_2R antagonists are summarized in Table 3.2. To get insights into the SAR, several modifications of the BIIE 0246 core structure were performed, both in the N-terminal part of the argininamide ("western part") and at the C-terminus ("eastern part"). Furthermore, an inversion of the (*S*)-arginine into the (*R*)-configuration was explored. Surprisingly, the (*R*)-argininamide ((*R*)-**3.47**) only shows a 5-fold lower affinity compared to the parent compound BIIE 0246 ((*S*)-**3.47**). This indicates a weak preference of the Y_2R for the (*S*)-configured arginine moiety, in contrast to the Y_1R, which clearly favors the (*R*)-enantiomers of antagonists derived from BIBP 3226.[31]

The replacement of the cyclopentan-1,1-diyl moiety by a methylene group in the "western part" of (*S*)-**3.59** resulted in a moderate decrease in affinity (K_i = 96 nM), verifying that this hydrophobic space-filling substructure confers to some extent to receptor binding. The relation between binding affinity K_i and the Gibbs free energy ($\Delta G = RT\ln K_i$) may serve as explanation. The released Gibbs free energy upon ligand binding results from enthalpic and entropic contributions ($\Delta G = \Delta H - T\Delta S$). The cyclo-aliphatic moiety in BIIE 0246 results in a rigidization of the molecule and therefore, in a lower decrease in conformational entropy upon binding to the Y_2R. Furthermore, the desolvation entropy is increased due to an additional hydrophobic effect of the cyclopentyl moiety.[32]

In compounds **3.46** and **3.61** the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione moiety was replaced by less lipophilic tyrosinamide and phenylalaninamide residues, respectively, strongly reminiscent of the C-terminus of the endogenous ligand NPY. As claimed in the patent literature, this exchange does not strongly alter the antagonistic activity (factor of 4-5).[5-7] Surprisingly, the data obtained from the

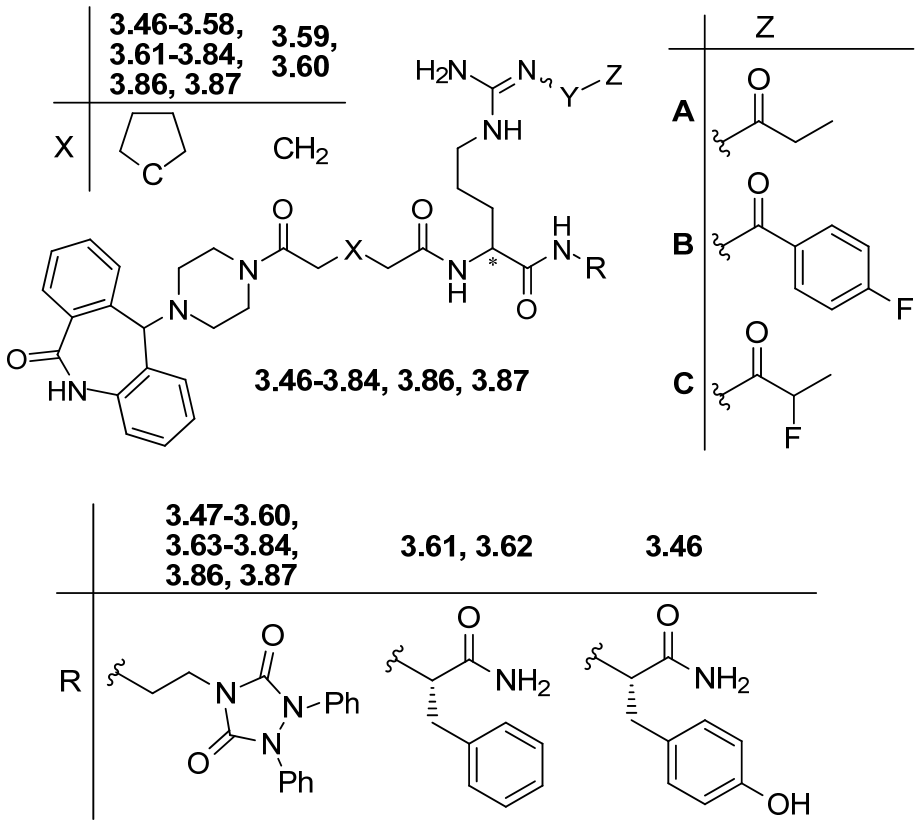
flow cytometric binding assay and the fura-2 Ca^{2+} assay revealed a different picture. The amides exhibited significantly lower Y_2R antagonistic activities and binding affinities than the reference compound BIIE 0246. Hence, it remains doubtful, whether BIIE 0246 and analogs are mimicking the C-terminus in NPY, in particular, because the peptide mainly interacts with extracellular parts of the Y_2R, whereas the smaller non-peptidic antagonists – according to mutagenesis studies – get access to the transmembrane domains of the receptor.[9, 11-13]

In previous studies, the exchange of the 5,11-dihydrodibenzo[*b*,*e*]azepin-6-one group by an α-diphenylmethyl residue led to a moderately decreased activity.[8] Nevertheless, such compounds were not further examined in the present work due to similar physicochemical properties and lack of obvious advantages compared to BIIE 0246.

In our laboratory the bioisosteric replacement of guanidine groups with acyl- or carbamoylguanidine moieties was developed and successfully applied to different classes of GPCR ligands.[16, 18, 20-21, 27, 33] Aiming at new pharmacological tools for the Y_2R, a small library of N^G-substituted amine precursors, varying in length and chemical nature of the spacer, were prepared to study the impact of the linker on affinity and selectivity. All derivatives with unmodified argininamide core structure ((*S*)-**3.48**-(*S*)-**3.58**) turned out to be highly potent Y_2R antagonists with retained, or even increased antagonistic activities and binding affinities regarding the unsubstituted BIIE 0246. Furthermore, the acylguanidine analogs exhibited lower retention times (t_R) compared to BIIE 0246, despite their reduced basicity in the N^G-position, due to an additional positive charge in the linker (representative results displayed in Table 3.3). Hence, these bioisosteres are considered as promising amine precursors for the synthesis of Y_2R tracers with improved physicochemical properties.

A correlation between linker length and affinity was obvious for the series of carbamoylated amine precursors (*S*)-**3.52**-(*S*)-**3.55** with linker lengths of 4-8 atoms. The N^G-carbamoylated analog (*S*)-**3.53** (n=5) revealed the highest binding affinity of all synthesized compounds (K_i = 2.1 nM). Nevertheless, length and chemical nature of the linker had only minor effects on Y_2R binding.

Table 3.2. Structures, Y_2R antagonistic activities (K_B) and affinities (K_i) of the guanidine-type Y_2R antagonists.



No	Y	Z	K_B / nM[a]	K_i / nM[b]
(*S*)-**3.47**[c]	H	--	5.6 ± 0.4	10.2 ± 1.1
(*R*)-**3.47**	H	--	56 ± 5	55 ± 3
(*S*)-**3.48**	$CO(CH_2)_3CONH(CH_2)_2NH_2$	--	3.7 ± 0.1	3.4 ± 0.1[d]
(*R*)-**3.48**	$CO(CH_2)_3CONH(CH_2)_2NH_2$	--	17 ± 6	36 ± 7
(*S*)-**3.49**	$CO(CH_2)_3CONH(CH_2)_3NH_2$	--	4.4 ± 0.5	2.3 ± 1.4
(*S*)-**3.50**	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH_2$	--	16 ± 2	16 ± 3
(*R*)-**3.50**	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH_2$	--	10 ± 2	42 ± 7
(*S*)-**3.51**	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3NH_2$	--	11 ± 3	16 ± 3[d]
(*S*)-**3.52**	$CONH(CH_2)_2NH_2$	--	7.0 ± 5.1	7.6 ± 4.2
(*S*)-**3.53**	$CONH(CH_2)_3NH_2$	--	2.5 ± 0.2	2.1 ± 0.3
(*S*)-**3.54**	$CONH(CH_2)_4NH_2$	--	1.8 ± 0.2	3.2 ± 0.3
(*S*)-**3.55**	$CONH(CH_2)_6NH_2$	--	2.1 ± 0.4	7.0 ± 1.2
(*S*)-**3.56**	$CO(CH_2)_5NH_2$	--	1.0 ± 0.2	7.8 ± 1.0[d]
(*S*)-**3.57**	$COCH_2(OCH_2CH_2)_2NH_2$	--	6.5 ± 0.02	18 ± 1
(*S*)-**3.58**	$CO(CH_2CH_2O)_4(CH_2)_2NH_2$	--	6.0 ± 0.5	8.1 ± 3.2
(*S*)-**3.59**	H	--	28 ± 3	96 ± 13
(*S*)-**3.60**	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH_2$	--	173 ± 27	141 ± 12
3.61	H	--	833 ± 275	496 ± 46
3.62	$CONH(CH_2)_3NH_2$	--	334 ± 124	191 ± 97

Table 3.2. (continued)

3.46	H	--	2200 ± 774	466 ± 3
(*S*)-**3.63**	$CO(CH_2)_3CONH(CH_2)_2NH$	A	16 ± 8	9.9 ± 1.0[d]
(*S*)-**3.64**	$CO(CH_2)_3CONH(CH_2)_3NH$	A	8.5 ± 0.1	22 ± 6
(*S*)-**3.65**	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	A	16 ± 8	9.9 ± 1.1
(*S*)-**3.66**	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3NH$	A	35 ± 15	37 ± 7[d]
(*S*)-**3.67**	$CONH(CH_2)_2NH$	A	30 ± 11	64 ± 4
(*S*)-**3.68**	$CONH(CH_2)_3NH$	A	22 ± 2	32 ± 0.1
(*S*)-**3.69**	$CONH(CH_2)_4NH$	A	9.4 ± 0.1	55 ± 15
(*S*)-**3.70**	$CONH(CH_2)_6NH$	A	12 ± 5	84 ± 64
(*S*)-**3.71**	$CO(CH_2)_5NH$	A	11 ± 4	9.0 ± 0.2[d]
(*S*)-**3.72**	$COCH_2(OCH_2CH_2)_2NH$	A	16 ± 2	15 ± 0.2
(*S*)-**3.73**	$CO(CH_2CH_2O)_4(CH_2)_2NH$	A	50 ± 3	48 ± 14
(*S*)-**3.74**	$CO(CH_2)_3CONH(CH_2)_2NH$	B	9.1 ± 1.8	8.3 ± 6.4[d]
(*S*)-**3.75**	$CO(CH_2)_3CONH(CH_2)_3NH$	B	11 ± 4	37 ± 7
(*S*)-**3.76**	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3NH$	B	15 ± 4	21 ± 9[d]
(*S*)-**3.77**	$CONH(CH_2)_3NH$	B	2.0 ± 2.0	24 ± 0.3
(*S*)-**3.78**	$CONH(CH_2)_4NH$	B	4.7 ± 0.8	68 ± 8
(*S*)-**3.79**	$CONH(CH_2)_6NH$	B	3.7 ± 0.1	82 ± 7
(*S*)-**3.80**	$CO(CH_2)_5NH$	B	8.4 ± 1.4	34 ± 6[d]
(*S*)-**3.81**	$COCH_2(OCH_2CH_2)_2NH$	B	13 ± 2	22 ± 9
(*S*)-**3.82**	$CO(CH_2CH_2O)_4(CH_2)_2NH$	B	26 ± 6	45 ± 3
(*S*)-**3.83**	$CO(CH_2)_3CONH(CH_2)_3NH$	C	12 ± 2	27 ± 4
(*S*)-**3.84**	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3NH$	C	27 ± 1	21 ± 1
3.86	--	A	8.2 ± 0.4	5.2 ± 1.8
3.87	--	B	14 ± 3	166 ± 65[d]

[a] Inhibition of 70 nM pNPY-induced $[Ca^{2+}]_i$ mobilization in CHO cells; mean values ± SEM (n = 2-5). [b] Flow cytometric binding assay using 5 nM Cy5-pNPY (K_D = 5.2 nM) in CHO cells, unless otherwise indicated; mean values ± SEM (n = 2-4). [c] BIIE 0246. [d] Dy-635-pNPY used as fluorescent ligand (10 nM) for competition binding.

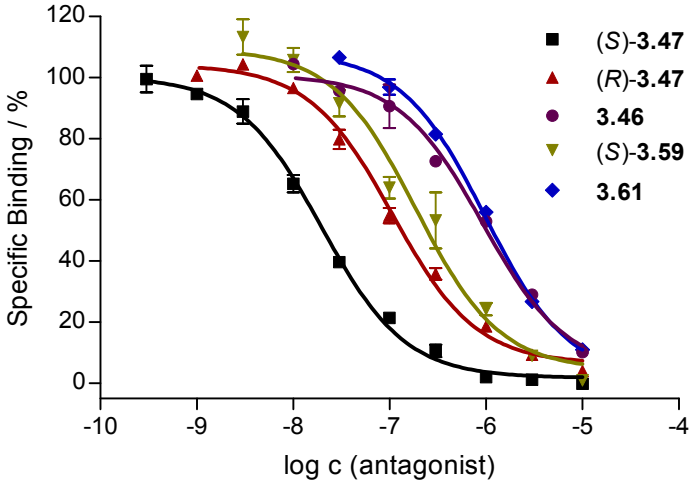


Figure 3.4. Flow cytometric competition binding assay using Cy5-pNPY as fluorescent ligand in the presence of N^G-unsubstituted Y_2R antagonists at various concentrations (mean values ± SEM, n = 2-3).

Table 3.3. Retention times (t_R) of the guanidine-type Y_2R antagonists (first row) and representative N^G-substituted acylguanidines (second and third row). Conditions: eluent: mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

No	t_R / min	No	t_R / min	No	t_R / min	No	t_R / min
(*S*)-**3.47**	18.25	(*S*)-**3.59**	14.27	**3.61**	14.91	**3.46**	11.94
(*S*)-**3.48**	14.69	(*S*)-**3.50**	12.99	(*S*)-**3.53**	14.97	(*S*)-**3.58**	15.39
(*S*)-**3.60**	11.80	**3.62**	13.06	(*S*)-**3.63**	17.04	(*S*)-**3.65**	14.55

All potential radioligands exhibited a decreased affinity compared to the corresponding amine precursors, except for compound (*S*)-**3.65**, which contains an additional, at physiological pH positively charged amine function in the linker (Figure 3.5a; highlighted in red). Acylguanidines derived from argininamides with lower affinity, comprising an ω-aminoacyllinker are superior in terms of binding and antagonism referred to their N^G-unsubstituted analogs ((*R*)-**3.48** and (*R*)-**3.50** vs. (*R*)-**3.47**; **3.62** vs. **3.61**). One exception within this series is antagonist (*S*)-**3.60**, comprising a second positive charge in the linker, with a two times lower affinity compared to (*S*)-**3.59**. Apparently, one additional polar group in the acyl linker of the antagonists is preferred by the Y_2R. Thus, "masking" the positive charge by acylation with propionic acid, 2-fluoropropionic acid, or 4-fluorobenzoic acid results in a decrease in affinity, especially in case of N^G-carbamoylated analogs ((*S*)-**3.67**-(*S*)-**3.70**, (*S*)-**3.77**-(*S*)-**3.79**). Maybe, the presence of the carbamoyl NH-group implicates a different orientation of the N^G-substituent which is less compatible with the acylation of the ω-amino residue. Nevertheless, most of the synthesized potential radioligands possess high affinities in the low nanomolar range, especially (*S*)-**3.63**, (*S*)-**3.65** and (*S*)-**3.71** with K_i values around 10 nM (Table 3.2). Direct propionylation in N^G-position yielded antagonist **3.86** with an increased binding affinity compared to BIIE 0246, whereas acylation with the 4-fluorobenzoyl substituent (**3.87**) resulted in a drastic decrease in binding, suggesting that a bulky substituent in proximity to the guanidine is less tolerated by the receptor. However, for a few compounds this affinity-lowering effect

was not reflected to the same extent by data from the fura-2 assay ((*S*)-**3.69**, (*S*)-**3.70**, (*S*)-**3.77**-(*S*)-**3.80**, **3.87**).

For most of the investigated compounds the binding data are in good agreement with the results from the functional assay. Discrepancies became obvious, in particular, regarding data for the ω-aminoacylated Y_2R antagonists consisting of a carbamoyl linker (cf. (*S*)-**3.67**-(*S*)-**3.70**, (*S*)-**3.77**-(*S*)-**3.79**). Depending on the structures and the binding kinetics of the investigated compounds, these discrepancies between K_i and K_B values presumably result from the different periods of incubation in the used assay types. The functional assays were performed in a time window of a few minutes, sufficient to reach equilibrium of the antagonists rather than the agonist pNPY, which is characterized by slow binding kinetics. By contrast, the flow cytometric binding assay was performed after incubation for 90 minutes.

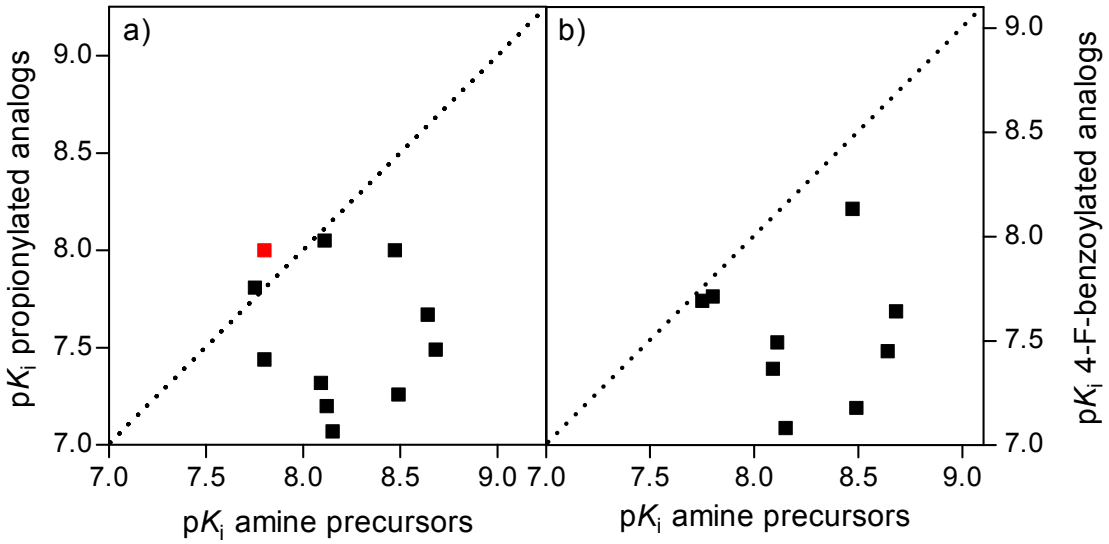


Figure 3.5. a) Comparison of binding data (pK_i) of the amine precursors (*S*)-**3.48**-(*S*)-**3.58** and the corresponding propionylated analogs (*S*)-**3.63**-(*S*)-**3.73** (highlighted in red: increased affinity of (*S*)-**3.65** vs. (*S*)-**3.50**). b) Comparison of binding data (pK_i) of the amine precursors (*S*)-**3.48**, (*S*)-**3.49**, (*S*)-**3.51**, (*S*)-**3.53**-(*S*)-**3.58** and the corresponding 4-fluorobenzoylated analogs (*S*)-**3.74**-(*S*)-**3.82**. pK_i values calculated from K_i values listed in Table 3.2. The straight dotted lines represent the theoretical correlations that would have been obtained if pK_i values had been identical for the compared analogs.

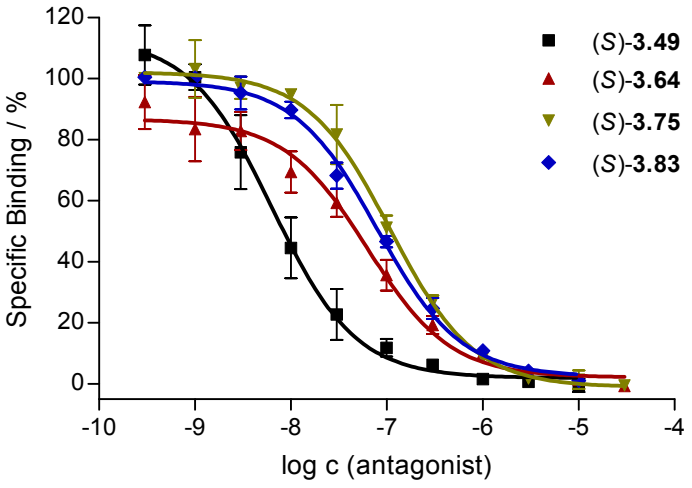


Figure 3.6. Displacement of Cy5-pNPY by N^G-substituted amine precursor (*S*)-**3.49** and the acylated analogs (*S*)-**3.64**, (*S*)-**3.75**, and (*S*)-**3.83** (mean values ± SEM; n = 2-3).

The Y_2R selectivity of representative examples of the argininamide-type Y_2R antagonists was confirmed by flow cytometric binding studies using Cy5-pNPY or Cy5-[K^4]hPP, respectively, and cells expressing human Y_1, Y_4 and Y_5 receptors (hY_1R, hY_4R, hY_5R) (Table 3.4). Hence, the structural changes in the pharmacophoric core structure and the bioisosteric replacement of the guanidine moiety, respectively, did not affect Y_2R selectivity.

Table 3.4. Binding data of selected Y_2R antagonists at hY_1R, hY_4R and hY_5R.

No	K_i / nM[a]			No	K_i / nM[a]		
	hY_1R	hY_4R	hY_5R		hY_1R	hY_4R	hY_5R
(*S*)-**3.47**	> 5000	> 7500	> 5000	(*S*)-**3.57**	> 3500	> 6500	> 5000
(*R*)-**3.47**	> 3500	> 7500	> 5000	(*S*)-**3.58**	> 3000	> 6500	> 5000
(*R*)-**3.48**	> 3500	> 6500	> 5000	**3.61**	> 3500	> 6500	> 500
(*S*)-**3.49**	> 5000	> 6500	> 5000	**3.46**	> 3500	> 6500	> 500
(*S*)-**3.50**	> 5000	> 6500	> 5000	(*S*)-**3.63**	> 5000	> 6500	> 5000
(*R*)-**3.50**	> 3500	> 6500	> 5000	(*S*)-**3.69**	> 3500	> 6500	> 5000
(*S*)-**3.53**	> 3000	> 6500	> 5000	(*S*)-**3.72**	> 3500	> 6500	> 5000
(*S*)-**3.54**	> 3000	> 6500	> 5000	(*S*)-**3.79**	> 3500	> 6500	> 2500
(*S*)-**3.55**	> 3500	> 6500	> 5000	(*S*)-**3.80**	> 3500	> 6500	> 5000
(*S*)-**3.56**	> 5000	> 6500	> 5000	**3.86**	> 3500	> 6500	> 5000

[a] Flow cytometric binding assays using 10 nM Cy5-pNPY (Y_1R), 5 nM Cy5-pNPY (Y_5R) or 3 nM Cy5-[K^4]-hPP (Y_4R) in HEL-Y_1 cells, CHO-Y_4 cells, and HEC-1B-Y_5 cells, respectively.

3.4 Summary and Conclusion

A variety of acylguanidine and guanidine-type analogs of the highly potent Y_2R antagonist BIIE 0246 was synthesized in order to elaborate the structure-activity relationships. Most compounds proved to be highly potent antagonists in the low nanomolar range. Modifications of the core structure of BIIE 0246 were performed in order to distinguish important structural motives for receptor binding. Surprisingly, the stereochemical switch from (*S*)- to (*R*)-configuration in the arginine moiety resulted in diastereomeres with hardly decreased antagonistic activity and binding affinity (factor 3-10). However, the affinity towards the Y_2R was considerably reduced (≈ 10-fold) when the cyclopentane-1,1-diyl substructure was replaced with a methylene group, proving that – to some extent – the more rigid and bulky moiety in the "western part" of BIIE 0246 confers to Y_2R binding. Since BIIE 0246 was developed as a putative mimic of the C-terminal part of NPY, the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione moiety was replaced by more hydrophilic groups, namely a

phenylalaninamide and a tyrosinamide moiety, respectively. These analogs showed drastically decreased affinities, in contrast to reported data from patent literature. Most likely these compounds do not mimic the endogenous peptide in terms of binding.

Additionally, N^G-substituted Y_2R antagonists bearing a free amine group were designed as precursors towards radio- and fluorescence labeled pharmacological tools following the guanidine-acylguanidine bioisosteric approach: the guanidine group was substituted with different ω-aminoacyl-, or ω-aminoalkylcarbamoyllinkers. Such derivatives – at physiological pH positively charged – proved to be highly potent Y_2R antagonists with retained or even increased affinity compared to the parent compound BIIE 0246. The N^G-carbamoylated ligand (*S*)-**3.53** is especially noteworthy as it represents the most potent, selective Y_2R antagonist known so far. Masking the positive charge in the linker, e.g., by acylation, has an affinity-lowering effect. Presumably, an additional positive charge in the linker contributes to Y_2R binding by electrostatic interactions.

Sufficient stability of the acylated and carbamoylated analogs in aqueous solution was proven for a selection of compounds. Acylguanidines containing a free amino group were less stable than the corresponding acylamino analogs. Y_2R antagonists containing a carbamoylguanidine moiety showed a very low degree of decomposition, demonstrating the superiority of carbamoylated compounds compared to acylated guanidines in terms of stability under assay conditions.

In conclusion, the acylguanidine substructure is useful for the synthesis of arginine-derived non-peptidic NPY Y_2R antagonists with reduced basicity. In view of recent results on Y_1R and histamine receptor ligands, our study corroborates the hypothesis that the guanidine-acylguanidine exchange is a promising and broadly applicable bioisosteric approach. As functionalization of the amine precursors is compatible with high Y_2R affinity and selectivity, these compounds are promising building blocks for the preparation of versatile pharmacological tools such as fluorescent and radiolabeled ligands.

3.5 Experimental Section

3.5.1 General Experimental Conditions

Unless otherwise stated, chemicals and solvents were purchased from commercial suppliers and used without further purification. *L*/*D*-Ornithine, *N*-*tert*-butoxycarbonyl-*L*-phenylalanine, and *N*-*tert*-butoxycarbonyl-*O*-benzyl-*L*-tyrosine were obtained from Iris Biotech (Marktredwitz, Germany). If not otherwise indicated, *tert*-butoxycarbonyl protected ω-amino carboxylic acids were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under argon atmosphere; DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Thin layer chromatography was performed on Merck silica gel 60 F_{254} TLC aluminum plates. Spots were visualized with UV light at 254 nm, and/or ninhydrin or ammonium molybdate/cerium (IV) sulfate solution. Geduran 60 (Merck, particle size 0.040-0.063 mm) was used for flash column chromatography. Flash chromatography was performed on an Intelli Flash 310 Flash Chromatography Workstation from Varian Deutschland GmbH (Darmstadt, Germany).

Optical rotations at 589 nm (Na_D line) were determined on an Optronic P8000T Polarimeter from A. Krüss (Hamburg, Germany) using a quartz micro-cuvette (layer thickness: 100 mm, volume: 1 mL, thermostated at 20 °C) and acetonitrile/H_2O 9:1 (v/v) as solvent. IR spectra were measured on a FTS 3000MX spectrometer (Excalibur Series) from Bio-Rad (Hercules, CA, USA) equipped with an attenuated total reflectance (ATR) unit (Specac Golden Gate Diamond Single Reflection ATR System).

NMR spectra were recorded on an Avance 300, an Avance 400 and an Avance III 600 spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany) with TMS as external standard. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), quat (quartet), p (quintet), m (multiplet), br (for broad singulet), as well as combinations thereof. Low resolution mass spectrometry analysis (MS) was performed in-house on a Finnigan ThermoQuest TSQ 7000 (ES-MS) and a Finnigan SSQ 710A (EI-MS 70 eV, CI-MS). High resolution mass spectrometry was performed on a Finnigan MAT 95 (LSI-MS).

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. The melting points are defined as the transition of the solid state into an amorphous state. Lyophilization was done with a Christ alpha 2-4 LD equipped with a vacuubrand RZ 6 rotary vane vacuum pump.

Preparative HPLC was performed with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector and a RP-column (Nucleodur 100-5 C18ec, 250 × 21 mm, 5 µm) at a flow rate of 22 mL/min. Mixtures of acetonitrile and 0.1 % aq. TFA were used as mobile phase. Acetonitrile was removed from the

eluates under reduced pressure (final pressure: 90 mbar) at 45 °C prior to lyophilization. Analytical HPLC analysis was performed on two different systems (Thermo Separation Products, Merck) which are listed in detail below. Mixtures of acetonitrile (A) and 0.05 % aq. TFA (B) (Thermo Separation Products), and mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B) (Merck), respectively, were used as mobile phase. Helium degassing was used throughout.
The buffer for the Y_2R binding studies on CHO cells was prepared by the addition of BSA (1 %) and bacitracin (100 µg/mL) to a buffer (pH 7.4) consisting of HEPES (25 mM), $CaCl_2 \times 2\ H_2O$ (2.5 mM), $MgCl_2 \times 6\ H_2O$ (1 mM). The loading buffer (pH 7.4) for the determination of the mobilization of intracellular Ca^{2+} in CHO cells was prepared by dissolving NaCl (120 mM), KCl (5 mM), $MgCl_2 \times 6\ H_2O$ (2 mM), $CaCl_2 \times 2\ H_2O$ (1.5 mM), HEPES (25 mM), and glucose (10 mM). Stock solutions of all compounds were prepared in DMSO at concentrations of 2 or 10 mM and stored at –20 °C.

Analytical HPLC systems:
System 1: Thermo Separation Products; composed of a SN400 controller, a P4000 pump, a degasser (Degassex DG-4400, phenomenex), an AS3000 autosampler and a Spectra Focus UV-VIS detector; flow rate: 0.8 mL/min; UV detection: 220 nm.
System 2: Merck; composed of a L-5000 controller, a 655A-12 pump, a 655A-40 autosampler and a L-4250 UV-VIS detector; flow rate: 0.7 mL/min; UV detection: 220 nm.

Applied gradients:
Gradient 1 (Thermo Separation Products): 0 to 30 min: A/B 20/80 to 95/5, 30 to 37 min: 95/5.
Gradient 2 (Merck): 0 to 30 min: A/B 20/80 to 95/5, 30 to 40 min: 95/5.
The purity of all compounds was determined with HPLC system 1 (gradient 1) on a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany), except for compounds (*R*)-**3.47**, (*R*)-**3.48**, (*R*)-**3.50**, **3.85**, which were analyzed with a Nucleodur H-Tec 100-5 C18 column (250 × 4 mm, 5 µm, Macherey-Nagel, Düren, Germany). The HPLC system 2 (gradient 2) was used for reaction monitoring with a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany).

3.5.2 Chemistry: Experimental Protocols and Analytical Data

(*R*)- and (*S*)-N^{δ}-Phthaloylornithine hydrochloride ((*R*)-**3.1**, (*S*)-**3.1**).[34] To a clear solution of (*D*)- or (*L*)-ornithine hydrochloride (21.91 g, 0.13 mol) and NaOH (10.4 g, 0.26 mol) in 200 mL water was added $CuSO_4 \times 5\ H_2O$ (16.25 g, 0.065 mol) in 200 mL water. After stirring for 15 min, $NaHCO_3$ (13.4 g, 0.16 mol) and *N*-ethoxycarbonyl phthalimide were added and the mixture was intensely stirred for an additional hour. The resulting blue copper complex of **3.1** was collected on a suction filter, washed with water, EtOH, $CHCl_3$ and diethyl ether, and dried in vacuum. To the finely grounded blue powder was added 6 M HCl (200 mL) and the green suspension was stirred for 1 h. The yellowish solid was separated by a suction filter, washed with 6 M HCl and air-dried. The crude product was dissolved in warm MeOH (450 mL) and filtered. The filtrate was diluted with ethyl acetate (900 mL) to obtain the product as a colorless crystalline precipitate. The product was collected on a filter, washed with ethyl acetate, and dried. A second crop of crystals was attained on concentration of the mother liquor (23.06 g; 60 %). Mp 223-224 °C (decomp.)[34]; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.79 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 3.59 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.87 (s, 1H, $C^{\alpha}\boldsymbol{H}$), 7.85 (m, 4H, Pht), 8.46 (br, 2H, $N\boldsymbol{H}_2$) ppm; MS (ES, +p) *m/z* (%): 263 (100) $[M+H]^+$, 217 (45) $[M+H-CH_2O_2]^+$; $C_{13}H_{14}N_2O_4 \times HCl$ (298.08).

(*R*)- and (*S*)-N^{α}-*tert*-Butoxycarbonyl-N^{δ}-phthaloylornithine ((*R*)-**3.2**, (*S*)-**3.2**).[35] (*R*)- or (*S*)-**3.1** (20.3 g, 68.0 mmol) was dissolved in 250 mL water and $NaHCO_3$ (12.60 g, 0.15 mol) was added in small portions. Subsequently, a solution of Boc_2O (15.27 g, 70.0 mmol) in 1,4-dioxane (700 mL) was introduced and the reaction mixture was stirred for 3 h. The solution was washed with diethyl ether (500 mL, discarded) and carefully adjusted to pH 2 with 2 M HCl. The product was extracted with ethyl acetate (3 × 250 mL); the combined extracts were dried over anhydrous Na_2SO_4 and the solvents were removed under reduced pressure. Recrystallization from toluene/petroleum ether gave the title compound as a white solid (14.62 g, 49 %). Mp. 126-127 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.35 (m, 9H, Boc), 1.47-1.78 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 3.57 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.85 (s, 1H, $C^{\alpha}\boldsymbol{H}$), 7.05 (m, 1H, N***H***), 7.84 (m, 4H, Pht), 12.40 (br, 1H, COO***H***) ppm; MS (ES, +p) *m/z* (%): 380 (100) $[M+NH_4]^+$, 363 (35) $[M+H]^+$; $C_{18}H_{22}N_2O_6$ (362.15).

1,2-Diphenyl-1,2,4-triazolidin-3,5-dione (3.3).[7] 1,2-Diphenylhydrazine (18.4 g, 0.1 mol) and ethyl allophanate (13.2 g, 0.1 mol) were heated in xylene (300mL) under reflux for 24 h. The mixture was allowed to cool to ambient temperature and then poured into 300 mL petroleum ether. The product was collected on a filter and re-dissolved in acetone (400 mL). Insoluble by-products were filtered off and the filtrate concentrated to about 200 mL. After addition of water (200 mL) crystals began to form. After complete crystallization the product was separated by filtration, washed with water, and dried in vacuum affording pale yellow crystals (14.0 g, 55 %). Mp 216-

217 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.22 (m, 2H, Ph), 7.37 (m, 8H, Ph), 12.05 (br, 1H, CON***H***CO) ppm; MS (CI, NH_3) *m/z* (%): 271 (100) $[M+NH_4]^+$, 254 (24) $[M+H]^+$; $C_{14}H_{11}N_3O_2$ (253.09).

4-(2-Phthalimidoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione.[7] **3.3** (10.12 g, 40 mmol) were dissolved in 120 mL DMF and the solution was cooled in an ice-water bath. KO^{t}Bu (4.94 g, 44 mmol) was added under stirring. After the KO^{t}Bu was completely dissolved, 2-bromoethylphthalimide (11.18 g, 44 mmol) was introduced and the mixture heated under reflux for 5 h. The resulting mixture was concentrated under reduced pressure and the residue was suspended in an aq. solution of K_2CO_3. The product was collected on a sintered filter, washed with water, and dried in vacuum at 50 °C. Recrystallization from 2-propanol afforded the title compound as a colorless, crystalline solid (10.31 g, 60 %). Mp 169-170 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.83 and 3.90 (m, 4H, C***H***$_2$C***H***$_2$), 7.23 (m, 2H, Ph), 7.32 (m, 8H, Ph), 7.80 (m, 4H, Pht) ppm; MS (CI, NH_3) *m/z* (%): 426 (100) [M]; $C_{24}H_{18}N_4O_4$ (426.13).

4-(2-Aminoethyl)-1,2-diphenyl-1,2,4-triazolidin-3,5-dione (3.4).[7] Phthalimide-protected precursor (10.28 g, 24.1 mmol) was dissolved in 200 mL THF/methanol 2:1 (v/v) and treated with hydrazine hydrate (3.31 g, 66.2 mmol). The mixture was stirred at ambient temperature for 16 h. 2 M HCl (45 mL) was added and stirring was continued for 2 h. The organic solvents were removed under reduced pressure and the residual suspension was diluted with water (120 mL) and alkalified with 2 M aq. NaOH (pH 10). The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL); the extracts were pooled, dried over K_2CO_3 and evaporated to dryness. The crude product was suspended in ethanol and treated with 1 M HCl in diethyl ether. The precipitate was collected on a filter, washed with petroleum ether, and dried. In order to obtain the free amine base, the hydrochloride was partitioned between 2 M aq. NaOH and CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhydrous K_2CO_3 and concentrated. The residue was dried in vacuum and recrystallized from hot cyclohexane to obtain the title compound as a white solid (5.28 g, 74 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.68 (br, 2H, N***H***$_2$), 2.79 (t, ^3J = 6.5 Hz, 2H, CH_2C***H***$_2$$NH_2$), 3.54 (t, ^3J = 6.5 Hz, 2H, C***H***$_2$$CH_2NH_2$), 7.23 (m, 2H, Ph), 7.38 (m, 8H, Ph) ppm; MS (CI, NH_3) *m/z* (%): 297 (100) $[M+H]^+$; $C_{16}H_{16}N_4O_2$ (296.13).

5*H*-Dibenzo[*b,e*]azepin-6,11-dione (3.5). A mechanically stirred mixture of 9,10-anthraquinone (20.8 g, 0.1 mol), sodium azide (7.8 g, 0.12 mol) and $CHCl_3$ (200 mL) was cooled in an ice-bath, while concentrated sulfuric acid (60 mL) was added dropwise. After completion of the addition the mixture was heated under reflux for 10 h. The cold mixture was poured into a chilled sodium carbonate solution (10 % w/w, 750 mL) in small portions. The mixture was alkalized with concentrated ammonia solution and allowed to stand until the precipitate had settled. The

supernatant clear solution was decanted and replaced by methanol (300 mL). The raw product was collected on a suction filter, washed with methanol and ether and dissolved in boiling acetic acid. Insoluble by-products were removed by filtering the hot suspension. After cooling, the product was separated by filtration and dried in vacuum at 60 °C affording fine, pale yellow crystals (13.2 g, 59 %). Mp 244-245 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.23 (m, 1H, ***H***$_{ar}$), 7.33 (m, 1H, ***H***$_{ar}$), 7.60 (m, 1H, ***H***$_{ar}$), 7.71 (m, 1H, ***H***$_{ar}$), 7.81 (m, 3H, ***H***$_{ar}$), 8.17 (m, 1H, ***H***$_{ar}$), 11.09 (br, 1H, N***H***) ppm; $C_{14}H_9NO_2$ (223.23).

11-Hydroxy-5,11-dihydrodibenzo[*b,e*]azepin-6-one (3.6). $NaBH_4$ (3.0 g, 80 mmol) was slowly added to a suspension of **3.5** (9.0 g, 40 mmol) in ethanol (500 mL). After complete addition, the mixture was heated under reflux for 1 h. The mixture was concentrated in vacuum to about 100 mL, treated with ammonium chloride solution and neutralized with hydrochloric acid. The colorless precipitate was filtered, washed with water, methanol, and petroleum ether, and dried in vacuum (8.5 g, 94 %) yielding a white powder. Mp 247-248 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.65 (s, 1H, 11-H), 6.40 (s, 1H, O***H***), 7.0-8.0 (m, 8H, ***H***$_{ar}$), 10.5 (s, 1H, N***H***) ppm; $C_{14}H_{11}NO_2$ (225.24).

11-Chloro-5,11-dihydrodibenzo[*b,e*]azepin-6-one (3.7). To a suspension of **3.6** (8.5 g, 37.5 mmol) in $CHCl_3$ (200 mL) was added $SOCl_2$ (10 mL) and the reaction mixture was heated under reflux for 30 min. The mixture was concentrated under reduced pressure and treated with n-hexane (100 mL). The product was collected on a filter, washed with n-pentane and dried in vacuum to afford a white powder (8.8 g, 96 %). Mp 218-220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.68 (s, 1H, 11-H), 7.0-8.0 (m, 8H, ***H***$_{ar}$), 10.84 (s, 1H, N***H***) ppm; $C_{14}H_{10}ClNO$ (243.69).

11-Piperazin-1-yl-5,11-dihydrodibenzo[*b,e*]azepin-6-one (3.8).[36] A solution of **3.7** (7.47 g, 30.7 mmol) in 150 mL 1,4-dioxane was added slowly to a stirred solution of piperazine (13.22 g, 153.5) in 250 mL 1,4-dioxane. To complete the reaction, the mixture was heated under reflux for 30 min. After cooling, the solvent was removed under reduced pressure and the residue partitioned between water (150 mL) and $CHCl_3$ (100 mL). The aqueous phase was extracted with two additional portions of $CHCl_3$ (2 × 100 mL) and the combined extracts were washed with 5 % aq. K_2CO_3 solution and dried over anhydrous Na_2SO_4. After evaporation of the volatiles the raw product was purified by flash chromatography (eluent: CH_2Cl_2/MeOH 3:1 + 1 Vol% NEt_3). The purified product was collected as a white powder (8.32 g, 92 %). Mp > 260 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.98 (m, 4H, 2 × C***H***$_2$CH$_2$NH), 3.17 and 3.32 (m, 4H, 2 × CH$_2$C***H***$_2$NH), 4.15 (s, 1H, ArC***H***Ar), 7.03 (m, 2H, ***H***$_{ar}$), 7.21 (m, 1H, ***H***$_{ar}$), 7.37 (m, 3H, ***H***$_{ar}$), 7.46 (m, 1H, ***H***$_{ar}$), 7.67 (m, 1H, ***H***$_{ar}$), 10.30 (s, 1H, N***H***) ppm; MS (CI, NH_3) *m/z* (%): 294 (100) $[M+H]^+$; $C_{18}H_{19}N_3O$ (293.15).

2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetic acid (3.9).[7] **3.8** (6.4 g, 21.8 mmol) in 80 mL anhydrous CH_2Cl_2 was slowly added to a stirred and chilled (0 °C) solution of 3,3-tetramethyleneglutaric anhydride (4.1 g, 24.0 mmol) in 35 mL anhydrous CH_2Cl_2. After stirring overnight at ambient temperature the reaction mixture was filtered and the filtrates were concentrated to dryness. Purification by flash chromatography (eluent: CH_2Cl_2 (A), MeOH (B); gradient: 0 to 4 min: A/B 100/0 to 96/4, 4 to 20 min: 96/4 to 90/10) yielded the product as a white powder (7.55 g, 75 %). Mp 153-157 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.53 (m, 8H, cyclopentyl), 2.04 (m, 4H, 3/5-H piperazine), 2.41 (s, 2H, >NC(O)C***H***$_2$), 2.45 (s, 2H, C***H***$_2$CO$_2$H), 3.18 (m, 4H, 2/6-H piperazine), 4.24 (s, 1H, dibenzazepine-11-yl), 7.06 (m, 2H, ***H***$_{ar}$), 7.24 (m, 1H, ***H***$_{ar}$), 7.32 (m, 1H, ***H***$_{ar}$), 7.40 (m, 2H, ***H***$_{ar}$), 7.49 (m, 1H, ***H***$_{ar}$), 7.72 (m, 1H, ***H***$_{ar}$), 10.35 (s, 1H, N***H***), 11.97 (s, 1H, CO$_2$***H***) ppm; MS (ES, +p) *m/z* (%): 462 (100) [*M*+H]$^+$; $C_{27}H_{31}N_3O_4$ (461.23).

5-Oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoic acid (3.10).[5] Preparation by analogy with the synthesis of **3.9** from **3.8** (1.92 g, 6.54 mmol) and glutaric anhydride (0.75 g, 6.54 mmol) in appropriate amounts of solvent. Purification by flash chromatography (eluent: CH_2Cl_2/MeOH 40/1 to 10/1) gave the title compound as a white powder (1.35 g, 51 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.65 (m, 2H, CH$_2$C***H***$_2$CH$_2$COOH), 2.04 (m, 4H, 3/5-H piperazine), 2.22 (m, 4H, C***H***$_2$CH$_2$C***H***$_2$COOH), 3.26 (m, 4H, 2/6-H piperazine), 4.25 (s, 1H, 11-H dibenzazepine-11-yl), 7.06 (m, 2H, ***H***$_{ar}$), 7.24 (m, 1H, ***H***$_{ar}$), 7.41 (m, 3H, ***H***$_{ar}$), 7.48 (m, 1H, ***H***$_{ar}$), 7.70 (m, 1H, ***H***$_{ar}$), 10.36 (s, 1H, NH lactam), 12.04 (br, 1H, COO***H***) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.1 (CH$_2$***C***H$_2$CH$_2$COOH), 31.2 ((CH$_2$)$_2$***C***H$_2$COOH), 32.8 (***C***H$_2$(CH$_2$)$_2$COOH), 40.8 (C-2/6 piperazine), 50.7 and 51.2 (C-3/5 piperazine), 73.7 (C-11 dibenzazepine-11-yl), 121.3 (***C***$_{ar}$H), 123.6 (***C***$_{ar}$H), 127.6 (***C***$_{ar}$H), 127.9 (***C***$_{ar}$H), 128.3 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.3 (***C***$_{ar}$H), 131.1 (***C***$_{ar}$H), 131.2 (***C***$_{ar}$), 131.6 (***C***$_{ar}$), 136.0 (***C***$_{ar}$), 142.0 (***C***$_{ar}$), 168.0 (>N***C***O), 169.9 (***C***=O lactam), 174.1 (***C***OOH) ppm; MS (ES, +p) *m/z* (%): 815 (34) [2*M*+H]$^+$, 408 (100) [*M*+H]$^+$; $C_{23}H_{25}N_3O_4$ (407.18).

(*R*)- and (*S*)-*N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\delta}$-phthaloylornithinamide ((*R*)-**3.11**, (*S*)-**3.11**).**[8] (*R*)- or (*S*)-**3.2** (5.07 g, 14.0 mmol) and HOBt × H_2O (2.22 g, 14.5 mmol) were dissolved in 50 mL THF and cooled to 0 °C. DCC (2.89 g, 14.0 mmol) was added in one batch, followed by **3.4** (4.80 g, 16.2 mmol). The reaction mixture was stirred and kept at 0 °C for 2 h; the cooling bath was removed and stirring was continued for 20 h. Dicyclohexylurea (DCU) was removed by filtration and the filtrate concentrated in vacuum. The residue was redissolved in ethyl acetate and washed with water, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Purification by flash chromatography (eluent: CH_2Cl_2/MeOH 80/1 to 40/1) gave the product as a white powder (8.13 g, 91 %). Mp 130 °C (decomp., sintering > 90 °C);

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.35 (m, 9H, Boc), 1.39-1.66 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 3.23-3.34 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.47 (m, 2H, NHC$\boldsymbol{H}_2CH_2$N<), 3.59 (m, 2H, NHCH$_2C\boldsymbol{H}_2$N<), 3.82 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 6.78 (d, ^3J = 8.0 Hz, 1H, N***H***), 7.22 (m, 2H, Ph), 7.38 (m, 8H, Ph), 7.85 (m, 4H, Pht), 8.11 (t, ^3J = 5.9 Hz, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 641 (100) [*M*+H]$^+$, 541 (45) [*M*+H–Boc]$^+$; $C_{34}H_{36}N_6O_7$ (640.69).

(*R*)- and (*S*)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{δ}-phthaloyl-ornithinamide·hydrochloride ((*R*)-**3.12**, (*S*)-**3.12).**[8] (*R*)- or (*S*)-**3.11** (7.00 g, 10.9 mmol) was dissolved in a saturated solution of HCl in ethyl acetate (25 mL). After stirring for 2 h the reaction mixture was poured into 300 mL of petroleum ether. The solid product was separated by filtration, washed with diethyl ether and n-pentane, and dried in vacuum to afford the title compound as a white solid (6.18 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.54-1.77 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 3.25-3.68 (m, 7H, $C^{\delta}\boldsymbol{H}_2$, NHC$\boldsymbol{H}_2C\boldsymbol{H}_2$N, $C^{\alpha}\boldsymbol{H}$), 7.23 (m, 2H, Ph), 7.39 (m, 8H, Ph), 7.87 (m, 4H, Pht), 8.21 (br, 3H, N$\boldsymbol{H}_3^+$), 8.83 (m, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 541 (100) [*M*+H]$^+$; $C_{29}H_{28}N_6O_5$ (540.21).

Preparation of N^{δ}-Pht protected ornithinamides (*R*)- and (*S*)-3.13 and (*S*)-3.14[8]

HOBt × H_2O (1 eq), the pertinent carboxylic acid (1 eq) and DIPEA (1 eq) were dissolved in acetonitrile and cooled to 0°C. EDC × HCl (1 eq) and subsequently a solution of the appropriate amine (1.15 eq) and DIPEA (1 eq) in acetonitrile were added. The reaction mixture was stirred for 16 h, during which time the cooling bath was allowed to come up to room temperature. The solvent was rotary evaporated and the residue was redissolved in CH_2Cl_2. The solution was washed with water, 5 % aq. $KHSO_4$, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude products were purified by flash chromatography.

(2*R*)- and (2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{δ}-phthaloylornithinamide ((*R*)-**3.13,** (*S*)-**3.13).** From **3.9** and amine (*R*)- or (*S*)-**3.12**. Purification by flash chromatography (eluent: CH_2Cl_2 (A), MeOH (B); gradient: 0 to 2 min: A/B 100/0 to 98/2, 2 to 15 min: 98/2 to 95/5, 15 to 25 min: 95/5) to yield a white solid (62-71 %). ^{1}H NMR (600 MHz, DMSO-d_6, COSY, HSQC): δ = 1.38 and 1.48 (m, 4H, 2/5-H cyclopentyl), 1.39 and 1.58 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.51 (m, 4H, 3/4-H cyclopentyl), 1.53 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.01 (m, 4H, 3/5-H piperazine), 2.20 (m, 2H, C$\boldsymbol{H}_2$CON$^{\alpha}$H), 2.44 (m, 2H, >NCOC$\boldsymbol{H}_2$), 3.24 and 3.26 (m, 4H, 2/6-H piperazine), 3.26 and 3.38 (m, 2H, NHC$\boldsymbol{H}_2$CH$_2$N<), 3.45 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.58 (m, 2H, NHCH$_2C\boldsymbol{H}_2$N<), 4.12 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.23 (m, 1H, 11-H dibenzazepin-11-yl), 7.03 (m, 1H, $\boldsymbol{H}_{ar}$), 7.06 (m, 1H, $\boldsymbol{H}_{ar}$), 7.20 (m, 2H, Ph), 7.24 (m, 1H, $\boldsymbol{H}_{ar}$), 7.32 (m, 1H, $\boldsymbol{H}_{ar}$), 7.35 (m, 8H, Ph), 7.38 (m, 1H, $\boldsymbol{H}_{ar}$), 7.39 (m, 1H, $\boldsymbol{H}_{ar}$), 7.46 (m, 1H, $\boldsymbol{H}_{ar}$), 7.70 (d, ^3J = 7.9 Hz, 1H, $\boldsymbol{H}_{ar}$), 7.82 (m,

2H, Pht), 7.84 (m, 2H, Pht), 7.92 (d, ^{3}J = 9.2 Hz, 1H, N$^{\alpha}$***H***), 8.16 (t, ^{3}J = 6.0 Hz, 1H, CON***H***$(CH_2)_2$), 10.32 (m, 1H, CON***H*** lactam) ppm; ^{13}C NMR (150 MHz, DMSO-d_6, HSQC): δ = 23.2 and 23.3 (C-3/4 cyclopentyl), 24.8 (***C***$^{\gamma}$), 29.1 (***C***$^{\beta}$), 36.1 (NH***C***H_2CH_2N<), 37.1 (***C***$^{\delta}$), 37.2 (C-2/5 cyclopentyl), 38.6 (>NCO***C***H_2-cyclopentyl), 39.6 (NHCH$_2$***C***H_2N<), 43.0 (***C***H_2CON$^{\alpha}$H), 44.0 (C-1 cyclopentyl), 45.3 and 45.5 (C-2/6 piperazine), 50.7 and 51.2 (C-3/5 piperazine), 51.9 (***C***$^{\alpha}$), 73.7 (C-11 dibenzazepine-11-yl), 121.3 (***C***$_{ar}$H), 122.6 (Ph), 123.0 (Pht), 123.7 (***C***$_{ar}$H), 126.6 (Ph), 127.7 (***C***$_{ar}$H), 127.9 (***C***$_{ar}$H), 128.4 (***C***$_{ar}$H), 129.0 (Ph), 129.8 (***C***$_{ar}$H), 130.4 (***C***$_{ar}$H), 131.3 (***C***$_{ar}$H), 131.7 (***C***$_{ar}$), 134.3 (Pht), 136.1 (***C***$_{ar}$), 136.6 (***C***$_{ar}$), 142.1 (***C***$_{ar}$), 152.6 (N(***C***ONPh)$_2$), 167.8 (***C***=O Pht), 168.1 (***C***=O lactam), 169.9 (***C***ON<), 171.1 (***C***ON$^{\alpha}$H), 171.9 (***C***ONH$(CH_2)_2$N<) ppm; MS (ES, +p) *m/z* (%): 985 (100) $[M+H]^+$; $C_{56}H_{57}N_9O_8$ (983.43).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoyl}-*N*$^{\delta}$-phthaloyl-ornithinamide ((*S*)-**3.14).** From **3.10** and (*S*)-**3.12**. Purification by flash chromatography (eluent: CH_2Cl_2/MeOH 40/1 to 20/1) to yield a white solid (50 %). MS (ES, +p) *m/z* (%): 930 (100) $[M+H]^+$; $C_{52}H_{51}N_9O_8$ (929.39).

Synthesis of *N*$^{\delta}$ deprotected ornithinamides (*R*)- and (S)-3.15 and (*S*)-3.16[8]

1 eq of the corresponding phthalimide ((*R*)-**3.13**, (*S*)-**3.13**, **3.14**) was dissolved in methanol and treated with hydrazine hydrate (2.5 eq). After stirring for 16 h a 1 M aq. solution of $KHSO_4$ (5 eq) was added and stirring was continued for additional 3 h. The precipitated solid was filtered off and washed with methanol. The filtrates were pooled, and the solvent was removed under reduced pressure. The residue was made alkaline with 5 % aq. K_2CO_3 and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude products were purified by flash chromatography (eluent: CH_2Cl_2 + 2 Vol% NEt_3 (A), MeOH (B); gradient: 0 to 5 min: A/B 98/2 to 95/5, 5 to 15 min: 95/5 to 90/10, 15 to 30 min: 90/10) yielding slightly yellowish crystalline solids.

(2*R*)- and (2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}-cyclopentyl)acetyl]ornithinamide ((*R*)-**3.15**, (*S*)-**3.15).** Yield: 71 %. ^{1}H NMR (600 MHz, DMSO-d_6, COSY, HSQC): δ = 1.20-1.31 (m, 2H, C$^{\gamma}$***H***$_2$), 1.35 and 1.58 (m, 2H, C$^{\beta}$***H***$_2$), 1.38 and 1.52 (m, 4H, 2/5-H cyclopentyl), 1.52 (m, 4H, 3/4-H cyclopentyl), 2.03 (m, 4H, 3/5-H piperazine), 2.20 and 2.24 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.40 (m, 2H, C$^{\delta}$***H***$_2$), 2.44 and 2.47 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.23 and 3.42 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.26 and 3.29 (m, 4H, 2/6-H piperazine), 3.58 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.08 (m, 1H, C$^{\alpha}$***H***), 4.22 (s, 1H, 11-H dibenzazepin-11-yl), 7.04 (m, 1H, ***H***$_{ar}$), 7.06 (m, 1H, ***H***$_{ar}$), 7.21 (m, 2H, Ph), 7.22 (m, 1H, ***H***$_{ar}$), 7.31 (m, 1H, ***H***$_{ar}$), 7.37 (m, 8H, Ph), 7.38 (m, 2H, ***H***$_{ar}$), 7.46 (m, 1H,

H$_{ar}$), 7.70 (d, ^{3}J = 7.6 Hz, 1H, ***H***$_{ar}$), 7.93 (d, ^{3}J = 8.1 Hz, 1H, N$^{\alpha}$***H***), 8.18 (t, ^{3}J = 6.0 Hz, 1H, CON***H***$(CH_2)_2$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6, HSQC): δ = 23.2 and 23.3 (C-3/4 cyclopentyl), 29.2 (***C***$^{\beta}$), 29.3 (***C***$^{\gamma}$), 36.0 (NH***C***H_2CH_2N<), 37.1 and 37.3 (C-2/5 cyclopentyl), 38.5 (>NCO***C***H_2-cyclopentyl), 39.8 (NHCH$_2$***C***H_2N<), 41.0 (***C***$^{\delta}$), 42.8 (***C***H_2CON$^{\alpha}$H), 44.1 (C-1 cyclopentyl), 45.5 (C-2/6 piperazine), 50.8 and 51.2 (C-3/5 piperazine), 52.1 (***C***$^{\alpha}$), 73.7 (C-11 dibenzazepine-11-yl), 121.3 (***C***$_{ar}$H), 122.6 (Ph), 123.7 (***C***$_{ar}$H), 126.6 (Ph), 127.6 (***C***$_{ar}$H), 127.9 (***C***$_{ar}$H), 128.3 (***C***$_{ar}$H), 129.0 (Ph), 129.9 (***C***$_{ar}$H), 130.4 (***C***$_{ar}$H), 131.3 (***C***$_{ar}$H), 131.7 (***C***$_{ar}$), 136.1 (***C***$_{ar}$), 136.6 (***C***$_{ar}$), 142.1 (***C***$_{ar}$), 152.6 (N(***C***ONPh)$_2$), 168.1 (***C***=O lactam), 169.9 (***C***ON<), 171.1 (***C***ON$^{\alpha}$H), 172.4 (***C***ONH$(CH_2)_2$N<) ppm; MS (ES, +p) *m/z* (%): 854 (100) $[M+H]^+$; $C_{48}H_{55}N_9O_6$ (853.43).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoyl}ornithinamide ((*S*)-**3.16).** Yield: 60 %. ^{1}H NMR (400 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.41 (m, 2H, C$^{\gamma}$***H***$_2$), 1.41 and 1.66 (m, 2H, C$^{\beta}$***H***$_2$), 1.81 (m, 2H, >NCOCH$_2$C***H***$_2$CH$_2$CO), 2.09 and 2.13 (m, 4H, 3/5-H piperazine), 2.22 (t, ^{3}J = 7.1 Hz, 2H, >NCO$(CH_2)_2$C***H***$_2$CO), 2.31 (m, 2H, >NCOC***H***$_2(CH_2)_2$CO), 2.49 (t, ^{3}J = 6.5 Hz, 2H, C$^{\delta}$***H***$_2$), 3.29 and 3.37 (m, 4H, 2/6-H piperazine), 3.46 and 3.57 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.75 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.19 (m, 2H, C$^{\alpha}$***H***, 11-H dibenzazepin-11-yl), 7.11 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.26 (m, 1H, ***H***$_{ar}$), 7.31-7.41 (m, 11H), 7.48 (m, 1H, ***H***$_{ar}$), 7.78 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (100 MHz, CD_3OD, HSQC, HMBC): δ = 22.4 and 22.5 (>NCOCH$_2$***C***H_2CH$_2$CO), 29.5 (***C***$^{\gamma}$), 30.2 (***C***$^{\beta}$), 33.3 (>NCO***C***$H_2(CH_2)_2$CO), 35.8 (>NCO$(CH_2)_2$***C***H_2CO), 38.4 (NH***C***H_2CH_2N<), 41.4 (NHCH$_2$***C***H_2N<), 41.8 (***C***$^{\delta}$), 42.8 and 46.9 (C-2/6 piperazine), 52.1 and 52.7 (C-3/5 piperazine), 54.5 (***C***$^{\alpha}$), 75.9 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.2 (Ph), 126.0 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.3 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 131.1 (***C***$_{ar}$H), 131.7 (***C***$_{ar}$H), 132.5 (***C***$_{ar}$), 133.0 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.9 (***C***$_{ar}$), 144.3 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 172.2 (***C***=O lactam), 173.2 (***C***ON<), 175.0 (***C***ONH$(CH_2)_2$N<), 175.5 (***C***ON$^{\alpha}$H) ppm; MS (ES, +p) *m/z* (%): 800 (50) $[M+H]^+$, 401 (100) $[M+2H]^{2+}$; $C_{44}H_{49}N_9O_6$ (799.38).

Synthesis of the amidated *N*$^{\alpha}$-Boc protected amino acids 3.17 and 3.18[23-24]

1 eq of the appropriate *N*$^{\alpha}$-Boc protected amino acid and NEt_3 (0.7 eq) were dissolved in anhydrous THF under argon atmosphere and cooled to 0 °C in an ice-water bath. IBCF was introduced dropwise and stirring at 0 °C was continued for 30 min before cooling to –78 °C. Then a 7 M solution of ammonia in MeOH was added and stirring was continued at –78 °C for 1 h and then at ambient temperature for an additional 16 h. After evaporation of the solvents, the residue was redissolved in ethyl acetate and washed with water. Subsequently, the aq. phase was extracted with ethyl acetate; the organic fractions were pooled, washed with 5 % aq. $KHCO_3$ and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude products were

subjected to flash chromatography (eluent: ethyl acetate) yielding the corresponding products as a white solid.

(*S*)-N^{α}-*tert*-Butoxycarbonylphenylalaninamide (3.17). Yield: 100 %. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.35 (s, 9H, Boc), 2.80 and 3.08 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 4.29 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 7.17-7.30 (m, 5H, Ph) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 28.7 ($C(\boldsymbol{C}H_3)_3$), 39.4 ($\boldsymbol{C}^{\beta}$), 57.2 ($\boldsymbol{C}^{\alpha}$), 80.6 ($\boldsymbol{C}(CH_3)_3$), 127.7 (C-4 aryl), 129.4 and 130.4 (C-2/3/5/6 aryl), 138.8 (C-1 aryl), 157.6 ($\boldsymbol{C}OOC(CH_3)_3$), 177.2 ($\boldsymbol{C}OOH$) ppm; MS (ES, +p) *m/z* (%): 265 (100) $[M+H]^+$; $C_{14}H_{20}N_2O_3$ (264.15).

(*S*)-N^{α}-*tert*-Butoxycarbonyl-*O*-benzyl-tyrosinamide (3.18). Yield: 97 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.30 (s, 9H, Boc), 2.65 and 2.86 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 4.02 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 5.05 (s, 2H, $OC\boldsymbol{H}_2$), 6.92 (d, ^{3}J = 8.5 Hz, 2H, Ph), 7.15 (d, ^{3}J = 8.5 Hz, 2H, Ph), 7.38 (m, 5H, Ph) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 28.1 ($C(\boldsymbol{C}H_3)_3$), 36.6 ($\boldsymbol{C}^{\beta}$), 55.7 ($\boldsymbol{C}^{\alpha}$), 69.0 ($O\boldsymbol{C}H_2$), 77.8 ($\boldsymbol{C}(CH_3)_3$), 114.2 (C-1 phenyl), 127.5 (Ph), 128.3 (Ph), 130.1 (Ph), 130.3 (Ph), 137.1 (C-1 benzyl), 155.1 ($\boldsymbol{C}OOC(CH_3)_3$), 156.7 (C-4 phenyl), 173.6 ($\boldsymbol{C}ONH_2$) ppm; MS (ES, +p) *m/z* (%): 741 (100) $[2M+H]^+$, 371 (60) $[M+H]^+$; $C_{21}H_{26}N_2O_4$ (370.19).

Preparation of the N^{α}-Boc deprotected amidated amino acids 3.19 and 3.20

Deprotection was carried out in TFA/CH_2Cl_2 1:1 (v/v) (approx. 5 mL/0.2 mmol substrate). After stirring for 2 h toluene was added and the volatiles were removed under reduced pressure. The products were used without further purification.

(*S*)-Phenylalaninamide (3.19).[37] Yield: 100 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.02 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 3.60 (br, 2H, $N\boldsymbol{H}_2$), 3.96 (t, ^{3}J = 6.5 Hz, 1H, $C^{\alpha}\boldsymbol{H}$), 7.21-7.38 (m, 5H, Ph), 8.18 (br, 2H, $CON\boldsymbol{H}_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 36.7 ($\boldsymbol{C}^{\beta}$), 53.3 ($\boldsymbol{C}^{\alpha}$), 127.0 (C-4 aryl), 128.4 and 129.4 (C-2/3/5/6 aryl), 134.5 (C-1 aryl), 169.6 ($\boldsymbol{C}ONH_2$) ppm; MS (ES, +p) *m/z* (%): 206 (100) $[M+H+CH_3CN]^+$, 165 (50) $[M+H]^+$; $C_9H_{12}N_2O$ (164.09).

(*S*)-*O*-Benzyl-tyrosinamide (3.20).[38] Yield: 100 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.95 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 3.90 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 5.08 (s, 2H, $OC\boldsymbol{H}_2$), 6.99 (d, ^{3}J = 8.6 Hz, 2H, Ph), 7.16 (d, ^{3}J = 8.6 Hz, 2H, Ph), 7.30-7.47 (m, 5H, Ph), 8.11 (br, 2H, $CON\boldsymbol{H}_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 35.9 ($\boldsymbol{C}^{\beta}$), 53.4 ($\boldsymbol{C}^{\alpha}$), 69.0 ($O\boldsymbol{C}H_2$), 114.7 (C-1 phenyl), 127.0 (Ph), 127.6 (Ph), 127.7 (Ph), 128.3 (Ph), 130.5 (Ph), 137.0 (C-1 benzyl), 157.4 (C-4 phenyl), 169.7 ($\boldsymbol{C}ONH_2$) ppm; MS (ES, +p) *m/z* (%): 541 (45) $[2M+H]^+$, 271 (100) $[M+H]^+$; $C_{16}H_{18}N_2O_2$ (270.14).

Preparation of N^{α}-Boc protected dipeptides 3.21 and 3.22

To a stirred solution of (*S*)-**3.2** (1 eq) and DIPEA (1 eq) in DMF was added TBTU (1 eq). The pertinent C-terminally amidated amino acid (1 eq) and DIPEA (1 eq) were introduced and stirring was continued for 16 h. The solution was diluted with ethyl acetate and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over Na_2SO_4 and evaporated. Flash chromatography (eluent: CH_2Cl_2/MeOH 40/1 to 20/1) afforded the products as white to yellow solids.

(*S*)-N^{α}-((*S*)-N^{α}-*tert*-Butoxycarbonyl-N^{δ}-phthaloylornithyl)phenylalaninamide (3.21). Yield: 77 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.34 (s, 9H, Boc), 1.48 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 2.82 and 2.96 (m, 2H, $C^{\beta}\boldsymbol{H}_2$ Phe-amide), 3.51 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.79 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Orn), 4.40 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Phe-amide), 7.00 (m, 1H, N***H***), 7.11 and 7.39 (m, 2H, CON$\boldsymbol{H}_2$), 7.18 (m, 5H, Ph), 7.67 (m, 1H, N***H***), 7.87 (m, 4H, Pht) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 24.8 ($\boldsymbol{C}^{\gamma}$), 28.0 (C($\boldsymbol{C}H_3)_3$), 37.0 ($\boldsymbol{C}^{\beta}$ Phe-amide), 37.4 ($\boldsymbol{C}^{\delta}$), 53.1 ($\boldsymbol{C}^{\alpha}$ Phe-amide), 78.1 ($\boldsymbol{C}(CH_3)_3$), 122.9 (Pht), 126.0 (C-4 aryl), 127.8 (Ph), 129.1 (Ph), 131.6 (Pht), 134.2 (C-1 aryl), 137.5 (Pht), 155.3 ($\boldsymbol{C}$OOC$(CH_3)_3$), 167.8 (***C***=O Pht), 171.4 ($\boldsymbol{C}ONH_2$), 172.5 ($\boldsymbol{C}ON^{\alpha}H$ Phe-amide) ppm; a peak for C^{α} and C^{β} Orn could not be detected; MS (ES, +p) *m/z* (%): 509 (100) $[M+H]^+$; $C_{27}H_{32}N_4O_6$ (508.23).

(*S*)-N^{α}-((*S*)-N^{α}-*tert*-Butoxycarbonyl-N^{δ}-phthaloylornithyl)-*O*-benzyl-tyrosinamide (3.22). Yield: 53 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.34 (s, 9H, Boc), 1.53 (m, 4H, $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 2.76 and 2.93 (m, 2H, $C^{\beta}\boldsymbol{H}_2$ Tyr(OBn)-amide), 3.52 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.79 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Orn), 4.35 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Tyr(OBn)-amide), 5.03 (s, 2H, OC$\boldsymbol{H}_2$), 6.88 (m, 2H, CON$\boldsymbol{H}_2$), 7.06 (m, 4H, Ph), 7.38 (m, 5H, Ph), 7.38 (m, 1H, N***H***), 7.62 (m, 1H, N***H***), 7.85 (m, 4H, Pht) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 24.9 ($\boldsymbol{C}^{\gamma}$), 28.0 (C($\boldsymbol{C}H_3)_3$), 36.0 ($\boldsymbol{C}^{\beta}$ Tyr(OBn)-amide), 37.0 ($\boldsymbol{C}^{\delta}$), 53.3 ($\boldsymbol{C}^{\alpha}$ Orn), 54.4 ($\boldsymbol{C}^{\alpha}$ Tyr(OBn)-amide), 69.0 (O$\boldsymbol{C}H_2$), 78.1 ($\boldsymbol{C}(CH_3)_3$), 114.1 (C-3/5 phenyl), 122.8 (Pht), 127.7 (C-3/5 benzyl), 128.3 (C-2/6 benzyl), 129.6 (C-2/6 phenyl), 130.2 (Pht), 131.6 (C-1 phenyl), 134.2 (Pht), 156.8 (C-4 phenyl), 167.9 (***C***=O Pht), 171.4 ($\boldsymbol{C}ONH_2$), 172.5 ($\boldsymbol{C}ON^{\alpha}H$ Tyr(OBn)-amide) ppm; a peak for C^{β} could not be detected; MS (ES, +p) *m/z* (%): 615 (100) $[M+H]^+$; $C_{34}H_{38}N_4O_7$ (614.27).

Preparation of the Boc-deprotected C-terminally amidated dipeptides 3.23 and 3.24

The pertinent dipeptide was dissolved in a saturated solution of HCl in ethyl acetate (25 mL). After stirring for 2 h the precipitate was filtered off; the mother liquor was poured into 300 mL of petroleum ether to give additional precipitated product. The solids were combined, washed with diethyl ether and n-pentane, and dried in vacuum to afford the title compounds as white solids.

(*S*)-N^{α}-((*S*)-N^{δ}-Phthaloylornithyl)phenylalaninamide (3.23). Yield: 82 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.69 (m, 4H, $C^{\beta}H_2C^{\gamma}H_2$), 2.85 and 2.96 (m, 2H, $C^{\beta}H_2$ Phe-amide), 3.56 (m, 2H, $C^{\delta}H_2$), 3.78 (m, 1H, $C^{\alpha}H$ Orn), 4.44 (m, 1H, $C^{\alpha}H$ Phe-amide), 7.10 and 7.60 (m, 2H, $CONH_2$), 7.19 (m, 1H, Ph), 7.26 (m, 4H, Ph), 7.86 (m, 4H, Pht), 8.18 (br, 2H, NH_2), 8.67 (m, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 23.6 (C^{γ}), 29.0 (C^{β} Orn), 37.4 (C^{β} Phe-amide), 52.4 (C^{α} Orn), 54.2 (C^{α} Phe-amide), 123.0 (Pht), 126.3 (C-4 aryl), 128.0 and 129.1 (C-2/3/5/6 aryl), 131.5 (Pht), 134.4 (C-1 aryl), 137.6 (Pht), 167.8 (*C*=O Pht), 172.1 ($CON^{\alpha}H$ Phe-amide) ppm; a peak for C^{δ} and $CONH_2$ could not be detected; MS (ES, +p) *m/z* (%): 409 (100) $[M+H]^+$; $C_{22}H_{24}N_4O_4$ (408.18).

(*S*)-N^{α}-((*S*)-N^{δ}-Phthaloylornithyl)-*O*-benzyl-tyrosinamide (3.24). Yield: 83 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.69 (m, 4H, $C^{\beta}H_2C^{\gamma}H_2$), 2.76 and 2.89 (m, 2H, $C^{\beta}H_2$ Tyr(OBn)-amide), 3.57 (m, 2H, $C^{\delta}H_2$), 3.75 (m, 1H, $C^{\alpha}H$ Orn), 4.41 (m, 1H, $C^{\alpha}H$ Tyr(OBn)-amide), 5.05 (s, 2H, OCH_2), 6.90 (m, 2H, Ph), 7.00-7.45 (m, 7H, Ph), 7.56 (m, 1H, NH), 7.88 (m, 4H, Pht), 8.05 (br, 3H, NH_3^+), 8.55 (m, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 23.6 (C^{γ}), 36.8 (C^{β} Tyr(OBn)-amide), 37.4 (C^{δ}), 51.7 (C^{α} Orn), 54.3 (C^{α} Tyr(OBn)-amide), 69.0 (OCH_2), 114.3 (C-3/5 phenyl), 123.0 (Pht), 127.5 (C-2/6 benzyl), 127.7 (C-4 benzyl), 128.3 (C-3/5 benzyl), 129.6 (C-2/6 phenyl), 130.1 (Pht), 131.5 (C-1 phenyl), 134.4 (Pht), 137.1 (C-1 benzyl), 156.9 (C-4 phenyl), 167.8 (*C*=O Pht), 172.2 ($CON^{\alpha}H$ Tyr(OBn)-amide) ppm; a peak for C^{β} could not be detected; MS (ES, +p) *m/z* (%): 515 (100) $[M+H]^+$; $C_{29}H_{30}N_4O_5$ (514.22).

General procedure for the preparation of N^{δ}-Pht protected ornithinamides 3.25 and 3.26

3.9 (1 eq) and DIPEA (1 eq) were dissolved in DMF. TBTU (1 eq) and subsequently the appropriate amine (1 eq) and DIPEA (1 eq) were added. After stirring for 16 h the reaction mixture was diluted with ethyl acetate and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude products were purified by flash chromatography (eluent: CH_2Cl_2/MeOH, 40/1 to 20/1) yielding white solids.

(2*S*)-N^{α}-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{δ}-phthaloylornithylphenylalaninamide (3.25). Yield: 81 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.40-1.60 (m, 12H, 2/5-H cyclopentyl, 3/4-H cyclopentyl, $C^{\beta}H_2C^{\gamma}H_2$), 2.03 (m, 4H, 3/5-H piperazine), 2.19 (m, 2H, $CH_2CON^{\alpha}H$), 2.41 (m, 2H, >NCOCH_2-cyclopentyl), 2.74 and 2.98 (m, 2H, $C^{\beta}H_2$ Phe-amide), 3.28 (m, 4H, 2/6-H piperazine), 3.49 (m, 2H, $C^{\delta}H_2$), 4.07 (m, 1H, $C^{\alpha}H$ Orn), 4.23 (br, 1H, 11-H dibenzazepine-11-yl), 4.34 (m, 1H, $C^{\alpha}H$ Phe-amide), 7.06 (m, 4H, Ph), 7.15 (m, 4H, Ph), 7.24 (m, 1H, H_{ar}), 7.31 (m, 1H, H_{ar}), 7.39 (m, 2H, Ph), 7.46 (m, 1H, H_{ar}), 7.46 and 7.70 (m, 2H, $CONH_2$), 7.85 (m, 4H, Pht), 7.92 (d, ^{3}J = 8.2 Hz, 1H, NH),

8.08 (m, 1H, N***H***), 10.36 (m, 1H, CON***H*** lactam) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 23.1 (C-3/4 cyclopentyl), 24.6 (***C***$^{\gamma}$), 28.9 (***C***$^{\beta}$ Orn), 37.0 (***C***$^{\delta}$), 37.0 (C-2/5 cyclopentyl), 37.3 (***C***$^{\beta}$ Phe-amide), 38.0 (>NCO***C***H_2-cyclopentyl), 42.6 (***C***H_2CON$^{\alpha}$H), 43.9 (C-1 cyclopentyl), 52.5 (***C***$^{\alpha}$ Orn), 53.6 (***C***$^{\alpha}$ Phe-amide), 73.6 (C-11 dibenzazepine-11-yl), 121.2 (***C***$_{ar}$H), 122.9 (Pht), 123.6 (***C***$_{ar}$H), 126.0 (Ph), 126.5 (Ph), 127.6 (***C***$_{ar}$H), 127.8 (***C***$_{ar}$H), 128.3 (***C***$_{ar}$H), 128.9 (Ph), 129.8 (***C***$_{ar}$H), 130.3 (***C***$_{ar}$H), 131.1 (Ph), 131.2 (***C***$_{ar}$H), 131.5 (***C***$_{ar}$), 134.3 (Pht), 136.0 (***C***$_{ar}$), 137.9 (Pht), 142.0 (***C***$_{ar}$), 167.7 (***C***=O Pht), 168.0 (***C***=O lactam), 169.9 (***C***ON<), 171.7 (***C***ON$^{\alpha}$H Orn), 172.7 (***C***ON$^{\alpha}$H Phe-amide) ppm; MS (ES, +p) *m/z* (%): 852 (100) [*M*+H]$^+$; $C_{49}H_{53}N_7O_7$ (851.40).

(2*S*)-*N*$^{\alpha}$-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]-*N*$^{\delta}$-phthaloylornithyl-*O*-benzyl-tyrosin-amide (3.26). Yield: 59 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.35-1.65 (m, 12H, 2/5-H cyclopentyl, 3/4-H cyclopentyl, C$^{\beta}$***H***$_2$C$^{\gamma}$***H***$_2$), 2.02 (m, 4H, 3/5-H piperazine), 2.20 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.40 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.70 and 2.92 (m, 2H, C$^{\beta}$***H***$_2$ Tyr(OBn)-amide), 3.26 (m, 4H, 2/6-H piperazine), 3.51 (m, 2H, C$^{\delta}$***H***$_2$), 4.11 (m, 1H, C$^{\alpha}$***H*** Orn), 4.21 (br, 1H, 11-H dibenzazepine-11-yl), 4.29 (m, 1H, C$^{\alpha}$***H*** Tyr(OBn)-amide), 5.02 (s, 2H, OC***H***$_2$), 6.85 (m, 2H, Ph), 7.07 (m, 5H, Ph), 7.21-7.43 (m, 10H), 7.48 and 7.71 (m, 2H, CON***H***$_2$), 7.83 (m, 4H, Pht), 7.83 (m, 1H, N***H***), 8.05 (m, 1H, N***H***), 10.37 (m, 1H, CON***H*** lactam) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 23.1 (C-3/4 cyclopentyl), 24.7 (***C***$^{\gamma}$), 29.0 (***C***$^{\beta}$ Orn), 37.0 (***C***$^{\delta}$), 37.0 (C-2/5 cyclopentyl), 37.3 (***C***$^{\beta}$ Tyr(OBn)-amide), 38.1 (>NCO***C***H_2-cyclopentyl), 42.6 (***C***H_2CON$^{\alpha}$H), 43.9 (C-1 cyclopentyl), 45.4 (C-2/6 piperazine), 50.7 (C-3/5 piperazine), 52.4 (***C***$^{\alpha}$ Orn), 53.8 (***C***$^{\alpha}$ Tyr(OBn)-amide), 69.0 (O***C***H_2), 73.6 (C-11 dibenzazepine-11-yl), 114.2 (C-3/5 phenyl), 121.2 (***C***$_{ar}$H), 122.9 (Pht), 123.6 (***C***$_{ar}$H), 127.5 (Ph), 127.7(***C***$_{ar}$H), 127.8 (***C***$_{ar}$H), 128.3 (Ph), 129.8 (***C***$_{ar}$H), 130.0 (Ph), 130.3 (***C***$_{ar}$H), 131.1 (Ph), 131.2 (***C***$_{ar}$H), 131.5 (***C***$_{ar}$), 134.3 (Pht), 136.0 (***C***$_{ar}$), 137.1 (Pht), 142.0 (***C***$_{ar}$), 156.8 (C-4 phenyl), 167.8 (***C***=O Pht), 168.1 (***C***=O lactam), 169.9 (***C***ON<), 171.3 (***C***ONH_2), 171.6 (***C***ON$^{\alpha}$H Orn), 172.7 (***C***ON$^{\alpha}$H Tyr(OBn)-amide) ppm; MS (ES, +p) *m/z* (%): 958 (100) [*M*+H]$^+$; $C_{56}H_{59}N_7O_8$ (957.44).

General procedure for the synthesis of *N*$^{\delta}$-Pht deprotected ornithinamides 3.27 and 3.28[8]

The compounds were prepared according to the experimental protocol for the synthesis of **3.15** and **3.16**. The crude products were purified by flash chromatography (eluent: CH_2Cl_2/MeOH + 1 Vol% NEt_3 10/1 to 3/1) yielding white solids.

(2*S*)-*N*$^{\alpha}$-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]ornithylphenylalaninamide (3.27). Yield: 84 %. ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.37 (m, 2H, C$^{\gamma}$***H***$_2$), 1.49 and 1.64 (m, 4H, 3/4-H cyclopentyl), 1.50 and 1.64 (m, 2H, C$^{\beta}$***H***$_2$ Orn), 1.50 and 1.72 (m, 4H, 2/5-H cyclopentyl), 2.15 and 2.19 (m, 4H, 3/5-H piperazine), 2.25 and 2.45 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.58 (m, 2H, C$^{\delta}$***H***$_2$), 2.87 and 3.16 (m, 2H, C$^{\beta}$***H***$_2$ Phe-amide), 3.37-3.58 (m, 4H, 2/6-H piperazine), 4.15 (m, 1H, C$^{\alpha}$***H*** Orn), 4.21 (m, 1H, 11-H dibenzazepine-11-yl), 4.56 (m, 1H, C$^{\alpha}$***H*** Phe-amide), 7.10 (m, 2H, ***H***$_{ar}$), 7.16 (m, 2H, ***H***$_{ar}$), 7.23 (m, 3H, ***H***$_{ar}$), 7.26 (m, 1H, ***H***$_{ar}$), 7.32 (m, 1H, ***H***$_{ar}$), 7.37 (m, 1H, ***H***$_{ar}$), 7.40 (m, 1H, ***H***$_{ar}$), 7.48 (m, 1H, ***H***$_{ar}$), 7.79 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 29.0 (***C***$^{\gamma}$), 29.9 (***C***$^{\beta}$ Orn), 38.3 and 38.4 (***C***$^{\beta}$ Phe-amide), 39.4 and 39.5 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.6 (***C***$^{\delta}$), 42.8 (C-2/6 piperazine), 44.5 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 52.1 and 52.6 (C-3/5 piperazine), 54.7 (***C***$^{\alpha}$ Orn), 55.8 (***C***$^{\alpha}$ Phe-amide), 75.8 (C-11 dibenzazepine-11-yl), 122.9 (***C***$_{ar}$H), 126.0 (***C***$_{ar}$H), 127.7 (***C***$_{ar}$H), 128.8 (***C***$_{ar}$H), 129.3 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.3 (***C***$_{ar}$H), 131.0 (***C***$_{ar}$H), 131.6 (***C***$_{ar}$H), 132.5 (***C***$_{ar}$), 133.0 (***C***$_{ar}$H), 133.5 (***C***$_{ar}$), 136.8 (***C***$_{ar}$), 138.9 (***C***$_{ar}$), 144.3 (***C***$_{ar}$), 172.2 (***C***=O lactam), 172.8 (***C***ON<), 174.3 (***C***ON$^{\alpha}$H Phe-amide), 175.3 (***C***ON$^{\alpha}$H Orn), 176.2 (***C***ONH$_2$) ppm; MS (ES, +p) *m/z* (%): 722 (95) [*M*+H]$^{+}$, 362 (100) [*M*+2H]$^{2+}$; $C_{41}H_{51}N_7O_5$ (721.40).

(2*S*)-*N*$^{\alpha}$-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]ornithyl-*O*-benzyl-tyrosinamide (3.28). Yield: 40 %. ^{1}H NMR (400 MHz, CD_3OD, HSQC): δ = 1.37 (m, 2H, C$^{\gamma}$***H***$_2$), 1.48 and 1.61 (m, 4H, 3/4-H cyclopentyl), 1.48 and 1.72 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.64 (m, 2H, C$^{\beta}$***H***$_2$ Orn), 2.13 (m, 4H, 3/5-H piperazine), 2.24 and 2.45 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.36 and 2.46 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.56 (m, 2H, C$^{\delta}$***H***$_2$), 2.82 and 3.10 (m, 2H, C$^{\beta}$***H***$_2$ Tyr(OBn)-amide), 3.32-3.56 (m, 4H, 2/6-H piperazine), 4.17 (m, 1H, 11-H dibenzazepine-11-yl), 4.18 (m, 1H, C$^{\alpha}$***H*** Orn), 4.51 (m, 1H, C$^{\alpha}$***H*** Tyr(OBn)-amide), 4.98 (m, 2H, OC***H***$_2$), 6.80 and 6.84 (m, 2H, 3/5-H phenyl), 7.09 (m, 2H, ***H***$_{ar}$), 7.14 (m, 2H, ***H***$_{ar}$), 7.32 (m, 9H, Ph), 7.46 (m, 1H, ***H***$_{ar}$), 7.79 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (100 MHz, CD_3OD, HSQC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 29.3 (***C***$^{\gamma}$), 29.9 (***C***$^{\beta}$ Orn), 37.6 and 37.7 (***C***$^{\beta}$ Tyr(OBn)-amide), 39.4 and 39.5 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.7 (***C***$^{\delta}$), 42.8 (C-2/6 piperazine), 44.5 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 52.2 and 52.7 (C-3/5 piperazine), 54.5 and 54.8 (***C***$^{\alpha}$ Orn), 55.8 (***C***$^{\alpha}$ Tyr(OBn)-amide), 71.0 (O***C***H$_2$), 75.8 (C-11 dibenzazepine-11-yl), 115.9 (C-3/5 phenyl), 122.9 (***C***$_{ar}$H), 125.9 (***C***$_{ar}$H), 128.5 (Ph), 128.9 (***C***$_{ar}$H), 129.3 (***C***$_{ar}$H), 129.5 (Ph), 129.8 (***C***$_{ar}$H), 131.0 (***C***$_{ar}$H), 131.4 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.0 (***C***$_{ar}$H), 133.4 (***C***$_{ar}$), 136.8 (***C***$_{ar}$), 138.7 (***C***$_{ar}$), 144.2 (***C***$_{ar}$), 159.0 (C-4 phenyl), 172.0 (***C***=O lactam), 172.7 (***C***ON<), 174.2 (***C***ON$^{\alpha}$H Tyr(OBn)-amide), 175.1 (***C***ON$^{\alpha}$H Orn), 176.1 (***C***ONH$_2$) ppm; MS (ES, +p) *m/z* (%): 828 (50) [*M*+H]$^{+}$, 415 (100) [*M*+2H]$^{2+}$; $C_{48}H_{57}N_7O_6$ (827.44).

tert*-Butyl (2-aminoethyl)-{2-[(*tert*-butoxycarbonyl)amino]ethyl}carbamate (3.29).**[39-40] A solution of ethyl trifluoroacetate (2.93 g, 20.6 mmol) in CH_2Cl_2 (24 mL) was added dropwise to a solution of diethylenetriamine (2.13 g, 20.6 mmol) in the same solvent (24 mL), while keeping the temperature at 0 °C. After complete addition, the mixture was stirred for 2 h at 0 °C and then for 2 h at rt. Removal of the solvent under reduced pressure yielded $H_2N(CH_2)_2NH(CH_2)_2NH(COCF_3)$ as a yellow oil. This compound was dissolved in CH_2Cl_2 (40 mL) and the resulting solution was cooled to –10 °C. Di-*tert*-butyl dicarbonate (9.0 g, 41.2 mmol) dissolved in CH_2Cl_2 (40 mL) was added dropwise to the cooled solution, and then the reaction mixture was stirred for 24 h at rt. After evaporation of the solvent, $(Boc)NH(CH_2)_2N(Boc)(CH_2)_2NH(COCF_3)$ was obtained as a dense oil, which was dissolved in a mixture of methanol (280 mL) and distilled water (12 mL). Then, K_2CO_3 (55.28 g, 0.4 mmol) was added and the mixture was refluxed for 2 h. After filtration, the solvent was removed under reduced pressure, the residue was redissolved in distilled water and the pH adjusted to pH = 13 with 40% aq. NaOH. Extraction with $CHCl_3$, followed by evaporation of the organic phase, gave the product as a yellow oil (3.80 g; 61 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.39 (s, 9H, Boc), 2.61 (m, 2H, CH***$_2$), 3.01 (m, 2H, C***H***$_2$), 3.14 (m, 4H, (C***H***$_2$)$_2$), 6.88 (br, 1H, N***H***) ppm; MS (CI, NH_3) *m/z* (%): 304 (61) $[M+H]^+$, 106 (100) $[M+H-2Boc]^+$; $C_{14}H_{29}N_3O_4$ (303.22).

5-{2-[(2-tert-Butoxycarbonylaminoethyl)(tert-butoxycarbonyl)amino]ethylamino}-5-oxopentanoic acid (3.30).[25] Compound **3.29** (1.00 g, 3.30 mmol) was dissolved in 5 mL THF and a solution of glutaric anhydride (0.38 g, 3.30 mmol) in 5 mL THF was added. Then NEt_3 (0.69 mL, 4.95 mmol) was added to the mixture. The mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the crude product was redissolved in 25 mL CH_2Cl_2, washed with 5 % aq. $KHSO_4$ (40 mL), dried over Na_2SO_4 and the solvent was evaporated giving a white solid (1.19 g, 86%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.39 (s, 9H, Boc), 1.68 (p, ^3J = 7.3 Hz, 2H, CH_2CH_2C***H***$_2$$CH_2$COOH), 2.07 (m, 2H, $(CH_2)_2$C***H***$_2$COOH), 2.19 (t, ^3J = 7.3Hz, 2H, C***H***$_2$$(CH_2)_2$COOH), 3.01 (m, 2H, BocNHC***H***$_2$), 3.15 (m, 6H, BocNHC***H***$_2$C***H***$_2$N(Boc)(C***H***$_2$)$_2$), 6.81 (br, 1H, N***H***Boc), 7.85 (br, 1H, N***H***), 12.02 (s, 1H, COO***H***) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.5 ($COCH_2$***C***H_2CH_2COOH), 25.0 (BocNH***C***H_2) 27.9 and 28.1 (C(***C***H_3)$_3$), 29.2 ($(CH_2)_2$***C***H_2COOH), 32.9 (***C***$H_2(CH_2)_2$COOH), 34.3 (CONH***C***H_2), 77.4 and 78.4 (***C***$(CH_3)_3$), 154.6 and 155.5 (***C***$OOC(CH_3)_3$), 171.5 (NH***C***$O(CH_2)_3$COOH), 174.1 (***C***OOH) ppm; MS (ES, +p) *m/z* (%): 853 (60) $[2M+NH_4]^+$, 418 (100) $[M+H]^+$; $C_{19}H_{35}N_3O_7$ (417.25).

Preparation of the carboxylic acids 3.31 and 3.32[41]

Glutaric anhydride (1 eq) was added to a solution of mono-Boc-protected ethane-1,2-diamine or mono-Boc-protected propane-1,3-diamine, respectively, in CH_2Cl_2 and the mixture was stirred for 2 h at rt. A 6-fold volume of CH_2Cl_2 was added prior to washing with a saturated solution of NH_4Cl (2-fold volume) and brine (2-fold volume). Drying over Na_2SO_4, filtration and removal of the solvent under reduced pressure yielded the products as highly viscous yellowish oils (95-97 %).

4-(2-*tert*-Butoxycarbonylaminoethyl)aminocarbonylbutanoic acid (3.31). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.42 (s, 9H, Boc), 1.88 (m, 2H, $NHCOCH_2C\boldsymbol{H}_2CH_2COOH$), 2.24 (t, ^3J = 7.4 Hz, 2H, $NHCOC\boldsymbol{H}_2CH_2CH_2COOH$), 2.32 (t, ^3J = 7.4 Hz, 2H, $NHCOCH_2CH_2C\boldsymbol{H}_2COOH$), 3.14 and 3.24 (m, 4H, $NHC\boldsymbol{H}_2C\boldsymbol{H}_2NHCO$) ppm; MS (CI, NH_3) *m/z* (%): 275 (100) $[M+H]^+$; $C_{12}H_{22}N_2O_5$ (274.31).

4-(3-*tert*-Butoxycarbonylaminopropyl)aminocarbonylbutanoic acid (3.32). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.47 (m, 2H, $NHCH_2C\boldsymbol{H}_2CH_2NHCO$), 1.69 (m, 2H, $NHCOCH_2C\boldsymbol{H}_2CH_2COOH$), 2.07 (t, ^3J = 7.4 Hz, 2H, $NHCOC\boldsymbol{H}_2CH_2CH_2COOH$), 2.19 (t, ^3J = 7.4 Hz, 2H, $NHCOCH_2CH_2C\boldsymbol{H}_2COOH$), 2.89 (quat, 2H, ^3J = 6.7 Hz, $NHC\boldsymbol{H}_2CH_2CH_2NHCO$), 3.00 (quat, 2H, ^3J = 6.7 Hz, $NHCH_2CH_2C\boldsymbol{H}_2NHCO$), 6.75 (m, 1H, N***H***), 7.76 (m, 1H, N***H***), 12.02 (s, 1H, COO***H***) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.6 ($NHCOCH_2\boldsymbol{C}H_2CH_2CO_2H$), 28.1 ($C(\boldsymbol{C}H_3)_3$), 29.5 ($NHCH_2\boldsymbol{C}H_2CH_2NHCO$), 32.9 ($NHCOCH_2CH_2\boldsymbol{C}H_2COOH$), 34.3 ($NHCO\boldsymbol{C}H_2CH_2CH_2COOH$), 36.0 ($NHCH_2CH_2\boldsymbol{C}H_2NHCO$), 37.5 ($NH\boldsymbol{C}H_2CH_2CH_2NHCO$), 77.3 ($\boldsymbol{C}(CH_3)_3$), 155.4 ($\boldsymbol{C}O(O)C(CH_3)_3$), 171.4 ($NH\boldsymbol{C}OCH_2CH_2CH_2COOH$), 174.0 ($NHCOCH_2CH_2CH_2\boldsymbol{C}OOH$) ppm; MS (CI, NH_3) *m/z* (%): 289 (100) $[M+H]^+$, 233 (50) $[M+H-C_4H_8]^+$; $C_{13}H_{24}N_2O_5$ (288.17).

6-(*tert*-Butoxycarbonylamino)hexanoic acid (3.33).[42] To a solution of 6-amino-hexanoic acid (1.97 g, 15 mmol) and $NaHCO_3$ (3.16 g, 37.5 mmol) in 30 mL H_2O was added di-*tert*-butyl dicarbonate (3.93 g, 18 mmol) in 30 mL 1,4-dioxane and stirred overnight at rt. The solution was washed with diethyl ether (30 mL) and the ether phase was discarded. The pH of the solution was adjusted to pH = 2 with 2 M HCl, washed with ethyl acetate (3 × 30 mL) and the organic phase was dried over Na_2SO_4. After filtration and evaporation of the solvent the product was obtained as a colorless solid (3.13 g, 90 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9H, Boc), 1.32-1.55 (m, 4H, $(CH_2)_2(C\boldsymbol{H}_2)_2CH_2NHBoc$), 1.75 (p, ^3J = 7.4 Hz, 2H, $CH_2C\boldsymbol{H}_2(CH_2)_3NHBoc$), 2.36 (t, ^3J = 7.4 Hz, 2H, $C\boldsymbol{H}_2(CH_2)_4NHBoc$), 3.12 (m, 2H, $(CH_2)_3C\boldsymbol{H}_2NHBoc$), 4.55 (br, 1H, N***H***Boc) ppm; MS (CI, NH_3) *m/z* (%): 232 (13) $[M+H]^+$, 193 (100) $[M+NH_4-C_4H_8]^+$; $C_{11}H_{21}NO_4$ (231.15).

Experimental details for the variously substituted *S*-methylisothioureas 3.34-3.45

N,N'*-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (3.34).**[43] *S*-Methylisothiouronium sulfate (3.02 g, 10.8 mmol) was dissolved in 60 mL water-dioxane 1:1 (v/v) and treated with 1 M aq. NaOH solution (20 mL) and Boc_2O (12.62 g, 53.8 mmol). After stirring for 16 h the precipitated product was filtered off and washed with a small amount of water; the filtrates were concentrated under reduced pressure to half the volume, and additional product was collected by filtration. The combined solid products were dried by lyophilization. Yield: 3.02 g (96 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.44 (s, 18H, 2x Boc), 2.28 (s, 3H, SCH***$_3$), 11.06 (br, 1H, N***H***Boc) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.7 (S***C***H_3), 27.6 (2x C(***C***$H_3)_3$), 164.9 (2x ***C***(O)OC$(CH_3)_3$) ppm; MS (CI, NH_3) *m/z* (%): 291 (100) $[M+H]^+$; $C_{12}H_{22}N_2O_4S$ (290.13).

S-Methylisothiourea and *N*-tert-butoxycarbonyl-*S*-methylisothiourea were prepared as previously described.[16, 25]

***N'*-Substituted *N*-tert-butoxycarbonyl-*S*-methylisothioureas (3.35-3.38, 3.43-3.45)**

TBTU (1 eq) was added to a solution of the respective carboxylic acid (1 eq), HOBt × H_2O (1 eq), DIPEA (1 eq) in DMF. *N*-tert-butoxycarbonyl-*S*-methylisothiourea (1 eq) was introduced and the solution was shaken for 16 h, diluted with ethyl acetate, washed with 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The crude product was dried over anhydrous Na_2SO_4 and evaporated to dryness. Purification by flash chromatography (eluent: CH_2Cl_2/ethyl acetate) afforded the title compounds.

Preparation of the guanidinylating agents 3.39-3.42

The succinimidyl ester of the corresponding carbamic acid (1.5 mmol) in DMF (1 mL) was added to a stirred solution of *N*-tert-butoxycarbonyl-*S*-methylisothiourea (0.29 g, 1.5 mmol) and DIPEA (0.51 mL, 3.0 mmol) in DMF (2 mL), and stirring was continued for 16 h. The solution was diluted with ethyl acetate (30 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4 and evaporated. The crude products were purified by flash chromatography (eluent: CH_2Cl_2/ethyl acetate 20:1 v/v).

N*-*tert*-Butoxycarbonyl-*N'*-[4-(2-*tert*-butoxycarbonylaminoethyl)aminocarbonyl-butanoyl]-*S*-methylisothiourea (3.35).** Yield: 64 %, as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9H, Boc), 1.52 (s, 9H, Boc), 2.00 (quat, ^3J = 7.2 Hz, 2H, $COCH_2C$H***$_2CH_2CO$), 2.26 (t, ^3J = 7.2 Hz, 2H, $CO(CH_2)_2C$***H***$_2CO$), 2.40 (s, 3H, SC***H***$_3$), 2.52 (t, ^3J = 7.0 Hz, 2H, COC***H***$_2(CH_2)_2CO$), 3.27 (m, 2H, $NHCH_2C$***H***$_2$NHBoc), 3.35 (m, 2H, NHC***H***$_2CH_2$NHBoc) ppm; MS (ES, +p) *m/z* (%):447 (100) $[M+H]^+$; $C_{19}H_{34}N_4O_6S$ (446.22).

***N-tert*-Butoxycarbonyl-*N'*-[4-(3-*tert*-butoxycarbonylaminopropyl)aminocarbonyl-butanoyl]-*S*-methylisothiourea (3.36).** Yield: 70 %, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9H, Boc), 1.51 (s, 9H, Boc), 1.61 (m, 2H, $NHCH_2C\boldsymbol{H}_2CH_2NHBoc$), 2.01 (m, 2H, $COCH_2C\boldsymbol{H}_2CH_2CO$), 2.27 (m, 2H, $CO(CH_2)_2C\boldsymbol{H}_2CO$), 2.39 (s, 3H, $SC\boldsymbol{H}_3$), 2.52 (m, 2H, $COC\boldsymbol{H}_2(CH_2)_2CO$), 3.16 (m, 2H, $NH(CH_2)_2C\boldsymbol{H}_2NHBoc$), 3.29 (m, 2H, $NHC\boldsymbol{H}_2(CH_2)_2NHBoc$) ppm; MS (CI, NH_3) *m/z* (%): 461 (100) $[M+H]^+$; $C_{20}H_{36}N_4O_6S$ (460.24).

N-tert*-Butoxycarbonyl-*N'*-{4-[2-(2-*tert*-butoxycarbonylaminoethyl)tert-butoxy-carbonylaminoethyl]aminocarbonylbutanoyl}-S-methylisothiourea (3.37).** Yield: 78 %, as yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.36 (s, 9H, Boc), 1.39 (s, 9H, Boc), 1.42 (s, 9H, Boc), 1.72 (p, ^{3}J = 7.3 Hz, 2H, $CH_2C\boldsymbol{H}_2CH_2CON=$), 2.27 (s, 3H, $SC\boldsymbol{H}_3$), 2.35 (m, 2H, $(CH_2)_2C\boldsymbol{H}_2CON=$), 2.69 (m, 2H, $C\boldsymbol{H}_2(CH_2)_2CON=$), 3.01 (m, 2H, $C\boldsymbol{H}_2NHBoc$), 3.15 (m, 6H, $BocNHCH_2C\boldsymbol{H}_2N(Boc)(C\boldsymbol{H}_2)_2$), 6.82 (br, 1H, NH***Boc), 7.86 (br, 1H, N***H***), 11.20 (br, 1H, N***H***Boc) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.0 ($S\boldsymbol{C}H_3$), 20.7 ($CH_2\boldsymbol{C}H_2CH_2CON=$), 27.6 and 27.9 and 28.1 ($C(\boldsymbol{C}H_3)_3$), 29.0 ($(CH_2)_2\boldsymbol{C}H_2CON=$), 29.9 ($\boldsymbol{C}H_2(CH_2)_2CON=$), 34.3 ($CONHCH_2\boldsymbol{C}H_2$), 39.7 ($CONH\boldsymbol{C}H_2$), 59.6 ($\boldsymbol{C}H_2N(Boc)\boldsymbol{C}H_2$), 77.4 and 78.4 and 80.0 ($\boldsymbol{C}(CH_3)_3$), 154.6 and 155.5 and 155.6 ($\boldsymbol{C}(O)OC(CH_3)_3$) ppm; MS (ES, +p) *m/z* (%): 590 (100) $[M+H]^+$; $C_{26}H_{47}N_5O_8S$ (589.31).

N*-tert-Butoxycarbonyl-*N*'-(18-tert-butoxycarbonylamino-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl)-*S*-methylisothiourea (3.38).** Yield: 58 %, as yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.42 (s, 9H, Boc), 1.59 (m, 4H, $OCH_2C\boldsymbol{H}_2CH_2NHBoc$ + $NHCH_2C\boldsymbol{H}_2CH_2O$), 2.27 (s, 3H, $SC\boldsymbol{H}_3$), 2.35 (m, 2H, $=NCOCH_2C\boldsymbol{H}_2CONH$), 2.56 (m, 2H, $=NCOC\boldsymbol{H}_2CH_2CONH$), 2.95 (m, 2H, $OCH_2CH_2C\boldsymbol{H}_2NHBoc$), 3.06 (m, 2H, $NHC\boldsymbol{H}_2CH_2CH_2O$), 3.38 (m, 4H, $((NH(CH_2)_2C\boldsymbol{H}_2O)_2$), 3.50 (m, 8H, $(O(C\boldsymbol{H}_2)_2O)_2$), 5.76 (s, 1H, $BocN\boldsymbol{H}C(SCH_3)=N$), 6.75 (m, 1H, NH***), 7.87 (m, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 593 (100) $[M+H]^+$; $C_{26}H_{48}N_4O_9S$ (592.31).

N-tert*-Butoxycarbonyl-*N'*-[*N*-(2-*tert*-butoxycarbonylaminoethyl)aminocarbonyl]-*S*-methylisothiourea (3.39).** Yield: 0.33 g (60 %, as colorless oil). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.44 (s, 9H, Boc), 2.28 (s, 3H, $SC\boldsymbol{H}_3$), 3.02 (m, 4H, $NHC\boldsymbol{H}_2C\boldsymbol{H}_2NHBoc$), 6.84 (m, 1H, NH***), 7.74 (m, 1H, N***H***), 12.33 (m, 1H, N***H***) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.5 ($S\boldsymbol{C}H_3$), 27.5 ($C(\boldsymbol{C}H_3)_3$), 28.1 ($C(\boldsymbol{C}H_3)_3$), 77.5 ($\boldsymbol{C}(CH_3)_3$), 82.0 ($\boldsymbol{C}(CH_3)_3$), 150.1 (***C***=O carbonyl), 155.5 (***C***=O carbonyl), 161.5 (***C***=N), 164.7 (***C***=O carbonyl) ppm; MS (ES, +p) *m/z* (%): 753 (40) $[2M+H]^+$, 377 (100) $[M+H]^+$; $C_{15}H_{28}N_4O_5S$ (376.18).

N*-*tert*-Butoxycarbonyl-*N'*-[*N*-(3-*tert*-butoxycarbonylaminopropyl)aminocarbonyl]-*S*-methylisothiourea (3.40).** Yield: 0.30 g (51 %, as colorless oil). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.44 (s, 9H, Boc), 1.53 (m, 2H, NHCH$_2$CH***$_2$CH$_2$NH), 2.28 (s, 3H, SC***H***$_3$), 2.91 and 3.03 (m, 2H, NHC***H***$_2$CH$_2$C***H***$_2$NH), 6.78 (m, 1H, N***H***), 7.75 (m, 1H, N***H***), 12.40 (br, 1H, N***H***) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.5 (S***C***H$_3$), 27.5 (C(***C***H$_3$)$_3$), 28.1 (C(***C***H$_3$)$_3$), 29.4 (NHCH$_2$***C***H$_2$CH$_2$NH), 54.8 (NH***C***H$_2$CH$_2$***C***H$_2$NH), 77.4 (***C***(CH$_3$)$_3$), 82.0 (***C***(CH$_3$)$_3$), 150.1 (***C***O(O)^{t}Bu), 155.5 (***C***O(O)^{t}Bu), 161.3 (N***C***(N)SCH$_3$), 164.7 (N***C***ONH) ppm; MS (ES, +p) *m/z* (%): 391 (100) [*M*+H]$^+$; $C_{16}H_{30}N_4O_5S$ (390.19).

N*-*tert*-Butoxycarbonyl-*N'*-[*N*-(4-*tert*-butoxycarbonylaminobutyl)aminocarbonyl]-*S*-methylisothiourea (3.41).** Yield: 0.30 g (48 %, as colorless oil); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.27-1.53 (m, 22H, 2 x Boc, CH$_2$(CH***$_2$)$_2$CH$_2$), 2.28 (s, 3H, SC***H***$_3$), 2.89 (m, 2H, C***H***$_2$NH), 3.01 (m, 2H, C***H***$_2$NH), 6.80 (t, ^3J = 5.2 Hz, 1H, CH$_2$N***H***), 7.79 (t, ^3J = 5.8 Hz, 1H, CH$_2$N***H***), 12.44 (s, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 405 (100) [*M*+H]$^+$; $C_{17}H_{32}N_4O_5S$ (404.21).

N*-*tert*-Butoxycarbonyl-*N'*-[*N*-(6-*tert*-butoxycarbonylaminohexyl)aminocarbonyl]-*S*-methylisothiourea (3.42).** Yield: 0.29 g (45 %, as yellow oil). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.23 (m, 4H, NH(CH$_2$)$_2$(CH***$_2$)$_2$(CH$_2$)$_2$NH), 1.32-1.48 (m, 22H, 2x Boc + NHCH$_2$C***H***$_2$(CH$_2$)$_2$C***H***$_2$CH$_2$NH), 2.28 (s, 3H, SC***H***$_3$), 2.88 (m, 2H, NHC***H***$_2$(CH$_2$)$_5$NH), 3.01 (m, 2H, NHC***H***$_2$(CH$_2$)$_5$NH), 6.77 (m, 1H, N***H***), 7.78 (m, 1H, N***H***), 12.43 (br, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 433 (100) [*M*+H]$^+$; $C_{19}H_{36}N_4O_5S$ (432.24).

N*-*tert*-Butoxycarbonyl-*N'*-[6-(*tert*-butoxycarbonylamino)hexanoyl]-*S*-methyl-isothiourea (3.43).** Yield: 85 %, as white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40-1.60 (m, 4H, (CH$_2$)$_2$(CH***$_2$)$_2$CH$_2$NHBoc), 1.44 (s, 9H, Boc), 1.52 (s, 9H, Boc), 1.70 (m, 2H, CH$_2$C***H***$_2$(CH$_2$)$_3$NHBoc), 2.40 (s, 3H, SC***H***$_3$), 2.45 (t, ^3J = 7.3 Hz, 2H, C***H***$_2$(CH$_2$)$_4$NHBoc), 3.12 (m, 2H, (CH$_2$)$_4$C***H***$_2$NHBoc), 4.56 (br, 1H, N***H***) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.5 (S***C***H$_3$), 24.4 (CH$_2$***C***H$_2$(CH$_2$)$_3$NHBoc), 26.2 (CH$_2$CH$_2$***C***H$_2$CH$_2$CH$_2$NHBoc), 28.0 (Boc), 28.4 (Boc), 29.8 ((CH$_2$)$_3$***C***H$_2$CH$_2$NHBoc), 37.1 (***C***H$_2$(CH$_2$)$_4$NHBoc), 40.4 ((CH$_2$)$_4$***C***H$_2$NHBoc), 77.2 (Boc), 79.1 (Boc), 156.0 (Boc), 169.3 (N***C***(SMe)N), 171.1 (***C***=O) ppm; MS (ES, +p) *m/z* (%): 404 (100) [*M*+H]$^+$, 304 (45) [*M*+H–Boc]$^+$; $C_{18}H_{33}N_3O_5S$ (403.54).

N*-*tert*-Butoxycarbonyl-*N'*-[8-(*tert*-butoxycarbonylamino-3,6-dioxa)octanoyl]-*S*-methylisothiourea (3.44).** Yield: 45 %, as yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9H, Boc), 1.45 (s, 9H, Boc), 2.30 (s, 3H, SCH***$_3$), 3.06 (quat, ^3J = 5.9 Hz, 2H, NHC***H***$_2$CH$_2$O), 3.39 (t, ^3J = 5.9 Hz, 2H, NHCH$_2$C***H***$_2$O), 3.57 and 3.65 (m, 4H, OC***H***$_2$C***H***$_2$O), 4.15 (s, 2H, =NCOC***H***$_2$O), 6.75 (m, 1H, N***H***), 12.22 (br, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 436 (100) [*M*+H]$^+$, 336 (25) [*M*+H–Boc]$^+$; $C_{18}H_{33}N_3O_7S$ (435.20).

N*-*tert*-Butoxycarbonyl-*N'*-[15-(*tert*-butoxycarbonylamino-4,7,10,13-tetraoxa)pentadecanoyl]-*S*-methylisothiourea (3.45).** Yield: 80 %, as slightly yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 and 1.43 (m, 18H, Boc), 2.28 (s, 3H, SCH***$_3$), 2.59 (t, ^{3}J = 5.8 Hz, 2H, NCOC***H***$_2$CH$_2$O), 3.05 (quat, ^{3}J = 5.9 Hz, 2H, NHC***H***$_2$CH$_2$O), 3.36 (m, 2H, NHCH$_2$C***H***$_2$O), 3.49 (m, 12H, (OC***H***$_2$C***H***$_2$O)$_3$), 3.63 (m, 2H, NCOCH$_2$C***H***$_2$O), 6.76 (m, 1H, N***H***) ppm; MS (ES, +p) *m/z* (%): 538 (100) [*M*+H]$^+$, 438 (19) [*M*+H–Boc]$^+$; $C_{23}H_{43}N_3O_9S$ (537.27).

General procedure for the guanidinylation with *S*-methylisothioureas (3.46a-3.62a)[16, 20, 25]

For the synthesis of **3.46a-3.62a** $HgCl_2$ (1 eq) was added to a solution of the corresponding ornithinamide (**3.15**, (*S*)-**3.16**, **3.27**, **3.28**) (1 eq), substituted *S*-methylisothiourea (1 eq), and NEt_3 (2 eq) in DMF (approx. 1 mL/0.1 mmol substrate). After stirring overnight the resulting mixture was diluted with ethyl acetate (10 mL/0.1 mmol substrate). Insoluble Hg-salts were separated by vacuum filtration through a short bed of Celite® in a Pasteur pipette plugged with some cotton wool and washed with ethyl acetate. The combined filtrates were washed with 5 % aq. $KHSO_4$ and brine and dried over anhydrous Na_2SO_4. The products were purified by flash chromatography (eluent: CH_2Cl_2/methanol 40/1 to 10/1).

(2*S*)-N^{ω},$N^{\omega'}$-Bis(*tert*-butoxycarbonyl)-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginyl-*O*-benzyltyrosinamide (3.46a). Yield: 84 %. MS (ES, +p) *m/z* (%): 1070 (25) [*M*+H]$^+$, 536 (100) [*M*+2H]$^{2+}$; $C_{59}H_{75}N_9O_{10}$ (1069.56).

(2*R*)- and (2*S*)-N^{ω},$N^{\omega'}$-Bis-*tert*-butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*R*)-**3.47a**, (*S*)-**3.47a).** Yield: 73 %. MS (ES, +p) *m/z* (%): 549 (100) [*M*+2H]$^{2+}$; $C_{59}H_{73}N_{11}O_{10}$ (1095.55).

(2*R*)- and (2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[4-(2-*tert*-butoxycarbonylaminoethyl)-aminocarbonylbutanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*R*)-**3.48a**, (*S*)-**3.48a).** Yield: 72 %. MS (ES, +p) *m/z* (%): 627 (100) [*M*+2H]$^{2+}$; $C_{66}H_{85}N_{13}O_{12}$ (1251.64).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[4-(3-*tert*-butoxycarbonylaminopropyl)aminocarbonylbutanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]-ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.49a)** Yield: 79 %. MS (ES, +p) *m/z* (%): 1266 (26) $[M+H]^+$, 634 (100) $[M+2H]^{2+}$; $C_{67}H_{87}N_{13}O_{12}$ (1265.66).

(2*R*)- and (2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-{4-[2-(2-tert-butoxycarbonylaminoethyl)-*tert*-butoxycarbonylaminoethyl]aminocarbonylbutanoyl}-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*R*)-**3.50a**, (*S*)-**3.50a).** Yield: 72 %. MS (ES, +p) *m/z* (%): 699 (100) $[M+2H]^{2+}$; $C_{73}H_{98}N_{14}O_{14}$ (1394.74).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-(18-*tert*-butoxycarbonylamino-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.51a).** Yield: 69 %. MS (ES, +p) *m/z* (%): 1399 (4) $[M+H]^+$, 700 (100) $[M+2H]^{2+}$; $C_{73}H_{99}N_{13}O_{15}$ (1397.74).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[*N*-(2-*tert*-butoxycarbonylaminoethyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.52a).** Yield: 43 %. MS (ES, +p) *m/z* (%): 1183 (20) $[M+H]^+$, 592 (100) $[M+2H]^{2+}$; $C_{62}H_{79}N_{13}O_{11}$ (1181.60).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[*N*-(3-*tert*-butoxycarbonylaminopropyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.53a).** Yield: 65 %. MS (ES, +p) *m/z* (%): 1197 (20) $[M+H]^+$, 599 (100) $[M+2H]^{2+}$; $C_{63}H_{81}N_{13}O_{11}$ (1195.62).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[*N*-(4-*tert*-butoxycarbonylaminobutyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.54a).** Yield: 83 %. MS (ES, +p) *m/z* (%): 1211 (100) $[M+H]^+$, 715 (20); $C_{64}H_{83}N_{13}O_{11}$ (1209.63).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[*N*-(6-*tert*-butoxycarbonylaminohexyl)amino-carbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclo-pentyl)acetyl]argininamide ((*S*)-**3.55a).** Yield: 72 %. MS (ES, +p) *m/z* (%): 1239 (100) $[M+H]^+$, 715 (30), 620 (33) $[M+2H]^{2+}$; $C_{66}H_{87}N_{13}O_{11}$ (1237.66).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[6-(*tert*-butoxycarbonylamino)hexanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginin-amide ((*S*)-**3.56a).** Yield: 86 %. MS (ES, +p) *m/z* (%): 1210 (50) $[M+H]^+$, 606 (100) $[M+2H]^{2+}$; $C_{65}H_{84}N_{12}O_{11}$ (1209.44).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[8-(*tert*-butoxycarbonylamino-3,6-dioxa)octanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*S*)-**3.57a).** Yield: 83 %. MS (ES, +p) *m/z* (%): 1242 (7) $[M+H]^+$, 621 (100) $[M+2H]^{2+}$; $C_{65}H_{84}N_{12}O_{13}$ (1240.63).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[15-(*tert*-butoxycarbonylamino-4,7,10,13-tetra-oxa)pentadecanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]-ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.58a).** Yield: 62 %. MS (ES, +p) *m/z* (%): 1366 (5) $[M+Na]^+$, 1344 (5) $[M+H]^+$, 683 (30) $[M+H+Na]^{2+}$, 672 (100) $[M+2H]^{2+}$; $C_{70}H_{94}N_{12}O_{15}$ (1342.70).

(2*S*)-N^{ω},$N^{\omega'}$-Bis(*tert*-butoxycarbonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin1-yl]pentanoyl}argininamide ((*S*)-**3.59a).** Yield: 83 %. MS (ES, +p) *m/z* (%): 1042 (20) $[M+H]^+$; 522 (100) $[M+2H]^{2+}$; $C_{55}H_{67}N_{11}O_{10}$ (1041.15).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-{4-[2-(2-*tert*-butoxycarbonylaminoethyl)*tert*-butoxycarbonylaminoethyl]aminocarbonylbutanoyl}-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoyl}argininamide ((*S*)-**3.60a).** Yield: 67 %. MS (ES, +p) *m/z* (%): 1342 (35) $[M+H]^+$; 672 (100) $[M+2H]^{2+}$; $C_{69}H_{92}N_{14}O_{14}$ (1340.69).

(2*S*)-N^{ω},$N^{\omega'}$-Bis(*tert*-butoxycarbonyl)-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginyl-phenylalaninamide (3.61a). Yield: 95 %. MS (ES, +p) *m/z* (%): 964 (35) $[M+H]^+$, 483 (100) $[M+2H]^{2+}$; $C_{52}H_{69}N_9O_9$ (963.52).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-N^{ω}-[*N*-(3-*tert*-butoxycarbonylaminopropyl)amino-carbonyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginylphenylalaninamide (3.62a). Yield: 83 %. MS (ES, +p) *m/z* (%): 1064 (30) $[M+H]^+$, 533 (100) $[M+2H]^{2+}$; $C_{56}H_{77}N_{11}O_{10}$ (1063.59).

(2*S*)-N^{α}-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginyltyrosinamide (3.46).[26] Compound **3.46a** (0.05 g, 0.05 mmol) was treated with a 1 M solution of TMSOTf (0.87 mL, 4.5 mmol), thioanisole (0.53 mL, 4.5 mmol), and m-cresole (0.05 mL, 0.5 mmol) in TFA for 90 min at ice-bath temperature. After evaporation of the solvents the crude product was purified using preparative HPLC to obtain the title compound as a white fluffy solid (40 mg, 90 %). Mp 180 °C; ^{1}H NMR (400 MHz, CD_3OD, HSQC): δ = 1.50 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.52 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.66 (m, 4H, 3/4-H cyclopentyl), 1.60 and 1.70 (m, 2H, $C^{\beta}\boldsymbol{H}_2$ Orn), 2.26 (m, 4H, 3/5-H piperazine), 2.28 and 2.49 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 2H, >NCOC$\boldsymbol{H}_2$-cyclopentyl), 2.80 and 3.07 (m, 2H, $C^{\beta}\boldsymbol{H}_2$ Tyr-amide), 3.11 (t, ^3J = 6.9 Hz, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.41 and 3.49 and 3.63 (m, 4H, 2/6-H piperazine), 4.22 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Orn), 4.33 (m, 1H, 11-H dibenzazepine-11-yl), 4.50 (m, 1H, $C^{\alpha}\boldsymbol{H}$ Tyr-amide), 6.60 and 6.66 (m, 2H, 3/5-H phenyl), 7.03 (m, 2H, $\boldsymbol{H}_{ar}$), 7.13 (m, 2H, 2/6-H phenyl), 7.29 (m, 1H, $\boldsymbol{H}_{ar}$), 7.34 (m, 1H, $\boldsymbol{H}_{ar}$), 7.41 (m, 2H, $\boldsymbol{H}_{ar}$), 7.51 (m, 1H, $\boldsymbol{H}_{ar}$), 7.83 (m, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (100 MHz, CD_3OD, HSQC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 26.0 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$ Orn), 37.8 and 37.9 ($\boldsymbol{C}^{\beta}$ Tyr-amide), 39.4 and 39.5 (C-2/5 cyclopentyl), 39.8 (>NCO$\boldsymbol{C}H_2$-cyclopentyl), 41.9 ($\boldsymbol{C}^{\delta}$), 42.5 (C-2/6 piperazine), 44.5 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 52.2 and 52.6 (C-3/5 piperazine), 54.2 ($\boldsymbol{C}^{\alpha}$ Orn), 55.9 ($\boldsymbol{C}^{\alpha}$ Tyr-amide), 75.8 (C-11 dibenzazepine-11-yl), 116.2 (C-3/5 phenyl), 122.9 (C-2/6 phenyl), 126.1 ($\boldsymbol{C}_{ar}H$), 129.6 ($\boldsymbol{C}_{ar}H$), 130.1 ($\boldsymbol{C}_{ar}H$), 131.4 ($\boldsymbol{C}_{ar}H$), 131.8 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.2 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.4 ($\boldsymbol{C}_{ar}$), 157.2 (C-4 phenyl), 158.6 (N$\boldsymbol{C}$(N)N), 171.9 ($\boldsymbol{C}$=O lactam), 172.9 ($\boldsymbol{C}$ON<), 173.8 ($\boldsymbol{C}ON^{\alpha}H$ Tyr-amide), 175.2 ($\boldsymbol{C}ON^{\alpha}H$ Orn), 176.1 ($\boldsymbol{C}ONH_2$) ppm; MS (ES, +p) *m/z* (%): 780 (30) $[M+H]^+$, 391 (100) $[M+2H]^{2+}$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{42}H_{54}N_9O_6$: 780.4197, found: 780.4211; RP-HPLC: 92 % (t_R = 12.2 min, k' = 3.5); $C_{42}H_{53}N_9O_6$ (779.41).

General procedure for the preparation of the deprotected argininamides 3.47-3.62[8]

Deprotection was carried out in TFA-CH_2Cl_2 1:1 (v/v) (approx. 5 mL/0.2 mmol substrate). After stirring for 2 h, CCl_4 or toluene (10 mL) was added, and the volatiles were removed under reduced pressure. The crude products were purified by preparative HPLC. The final products were obtained as white to slightly yellowish powders in yields from 70 to 99 %.

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*S*)-**3.47).** Mp 125 °C; $[\alpha]_D^{20}$= –3.3 cm^3 g^{-1} dm^{-1} (c = 0.66 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, DMSO-d_6, HSQC): δ = 1.44 (m, 2H, C$^{\gamma}$***H***$_2$), 1.40 and 1.65 (m, 2H, C$^{\beta}$***H***$_2$), 1.40-1.55 (m, 4H, 2/5-H cyclopentyl), 1.55 (m, 4H, 3/4-H cyclopentyl), 2.11 (m, 4H, 3/5-H piperazine), 2.24 and 2.30 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.46 and 2.52 (m, 2H, NCOC***H***$_2$-cyclo-C_5H_8), 3.00 (m, 2H, C$^{\delta}$***H***$_2$), 3.32 (m, 4H, 2/6-H piperazine), 3.29 and 3.38 (m, 2H, NHC***H***$_2$CH$_2$N), 3.59 (m, 2H, NHCH$_2$C***H***$_2$N), 4.14 (m, 1H, C$^{\alpha}$***H***), 4.30 (m, 1H, 11-***H*** dibenzazepin-11-yl), 7.03 (m, 1H, ***H***$_{ar}$), 7.11 (m, 1H, ***H***$_{ar}$), 7.21 (m, 2H, Ph), 7.22 (m, 1H, ***H***$_{ar}$), 7.32 (m, 1H, ***H***$_{ar}$), 7.37 (m, 8H, Ph), 7.38 (m, 2H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.73 (m, 1H, ***H***$_{ar}$), 8.02 (d, ^{3}*J* = 7.5 Hz, 1H, N$^{\alpha}$***H***), 8.32 (t, ^{3}*J* = 5.6 Hz, 1H, CON***H***C$_2$H$_4$N), 10.40 (s, 1H, C(O)N***H*** lactam) ppm; ^{13}C NMR (150 MHz, DMSO-d_6, HSQC): δ = 23.2 (***C***-3/4 cyclopentyl), 24.9 (***C***$^{\gamma}$), 28.6 (***C***$^{\beta}$), 36.0 (NH***C***H$_2$CH$_2$N), 37.1 (***C***-2/5 cyclopentyl), 38.5 (N***C***O***C***H$_2$-cyclo-C_5H_8), 39.6 (NHCH$_2$***C***H$_2$N), 40.1 (***C***$^{\delta}$), 41.9 and 45.5 (***C***-2/6 piperazine), 42.7 (***C***H$_2$CON$^{\alpha}$H), 43.9 (***C***-1 cyclopentyl), 50.7 (***C***-3/5 piperazine), 51.9 (***C***$^{\alpha}$), 73.7 (***C***-11 dibenzazepin-11-yl), 121.3 (***C***$_{ar}$H), 122.7 (Ph), 123.8 (***C***$_{ar}$H), 126.6 (Ph), 127.8 (2 ***C***$_{ar}$H), 128.3 (***C***$_{ar}$H), 128.9 (Ph), 129.7 (***C***$_{ar}$H), 130.4 (***C***$_{ar}$H), 131.1 (***C***$_{ar}$H), 131.4 (***C***$_{ar}$), 136.3 (***C***$_{ar}$), 136.4 (***C***$_{ar}$), 143.4 (***C***$_{ar}$), 152.5 (N***C***ONPh), 156.9 (N***C***(N)N), 168.2 (***C***=O lactam), 169.9 (***C***ON), 171.2 (***C***ON$^{\alpha}$H), 171.9 (***C***ONHC$_2$H$_4$N) ppm; IR (neat): υ´ = 3281, 2963, 1649, 1446, 1176, 1132 cm^{-1}; MS (ES, +p) *m/z* (%): 897 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd for $C_{49}H_{58}N_{11}O_6$: 896.4572, found: 896.4550; RP-HPLC: 100 % (t_R = 18.3 min, *k'* = 5.7); anal. calcd. for $C_{49}H_{57}N_{11}O_6$·4 H_2O·5 HCl: C 51.16, H 6.13, N 13.40 %, found: C 51.12, H 6.25, N 13.08 %; $C_{49}H_{57}N_{11}O_6$ (896.05).

(2*R*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*R*)-**3.47).** Mp 125 °C; $[\alpha]_D^{20}$= +4.1 cm^3 g^{-1} dm^{-1} (c = 0.88 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, CD_3OD, HSQC, HMBC): δ = 1.48 and 1.72 (m, 2H, C$^{\beta}$***H***$_2$), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.51 (m, 2H, C$^{\gamma}$***H***$_2$), 1.63 (m, 4H, 3/4-H cyclopentyl), 2.21 and 2.29 (m, 4H, 3/5-H piperazine), 2.26 and 2.42 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.47 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.02 (m, 2H, C$^{\delta}$***H***$_2$), 3.41-3.46 (m, 4H, 2/6-H piperazine), 3.47 and 3.53 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.23 (m, 1H, C$^{\alpha}$***H***), 4.41 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.84 (d, ^{3}*J* = 7.7 Hz, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 26.3 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.2 (NHCH$_2$***C***H$_2$N<), 41.9 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 53.9 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.2 (Ph), 126.1 (***C***$_{ar}$H), 128.2 (***C***$_{ar}$H), 129.4 (***C***$_{ar}$H), 129.7 (***C***$_{ar}$H), 130.2 (Ph), 131.4 (***C***$_{ar}$H),

131.9 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 158.6 (N***C***(N)N), 171.7 (***C***=O lactam), 172.8 (***C***ON<), 174.6 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (ES, +p) *m/z* (%): 897 (60) [*M*+H]$^+$, 449 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{49}H_{58}N_{11}O_6$: 896.4572, found: 896.4603; RP-HPLC: 98 % (t_R = 18.3 min, *k′* = 5.8); $C_{49}H_{57}N_{11}O_6$ (895.45).

(2*S*)-*N*$^{\omega}$-[4-(2-Aminoethyl)aminocarbonylbutanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-3.48). Mp 138 °C; $[\alpha]_D^{20}$= –7.6 cm^3 g^{-1} dm^{-1} (c = 0.98 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, CD_3OD, HSQC): δ = 1.51 and 1.75 (m, 2H, C$^{\beta}$***H***$_2$), 1.52 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.61 (m, 2H, C$^{\gamma}$***H***$_2$), 1.93 (p, ^3J = 7.4 Hz, 2H, COCH$_2$C***H***$_2$CH$_2$CO), 2.28 and 2.36 (m, 4H, 3/5-H piperazine), 2.29 and 2.44 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.30 (m, 2H, COC***H***$_2$(CH$_2$)$_2$CONH), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.51 (m, 2H, CO(CH$_2$)$_2$C***H***$_2$CONH), 3.04 (t, ^3J = 5.9 Hz, 2H, NHCH$_2$C***H***$_2$NH$_2$), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.34-3.55 (m, 4H, 2/6-H piperazine), 3.44 (t, ^3J = 5.9 Hz, 2H, NHC***H***$_2$CH$_2$NH$_2$), 3.45 and 3.54 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.51 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.43 (m, 1H, ***H***$_{ar}$), 7.47 (m, 1H, ***H***$_{ar}$), 7.54 (m, 1H, ***H***$_{ar}$), 7.87 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 21.0 (COCH$_2$***C***H$_2$CH$_2$CO), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 35.4 (CO***C***H$_2$(CH$_2$)$_2$CONH), 36.8 (CO(CH$_2$)$_2$***C***H$_2$CONH), 38.2 (NH***C***H$_2$CH$_2$NH$_2$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.9 (NHCH$_2$***C***H$_2$NH$_2$), 41.2 (NHCH$_2$***C***H$_2$N<), 41.9 (***C***$^{\delta}$), 44.6 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 46.7 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 130.0 (***C***$_{ar}$H), 130.2 (Ph), 131.6 (***C***$_{ar}$H), 132.2 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.5 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.1 (N***C***(N)N), 171.2 (***C***=O lactam), 172.8 (***C***ON<), 174.6 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H), 176.4 (CO(CH$_2$)$_3$***C***ONH), 176.7 (***C***O(CH$_2$)$_3$CONH) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1052 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{56}H_{70}N_{13}O_8$: 1052.5470, found: 1052.5462; RP-HPLC: 100 % (t_R = 14.7 min, *k´* = 4.4); $C_{56}H_{69}N_{13}O_8$ (1051.54).

(2*R*)-*N*$^{\omega}$-[4-(2-Aminoethyl)aminocarbonylbutanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*R*)-3.48). Mp 138 °C; $[\alpha]_D^{20}$= +5.6 cm^3 g^{-1} dm^{-1} (c = 0.57 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, CD_3OD, HSQC): δ = 1.51 and 1.75 (m, 2H, C$^{\beta}$***H***$_2$), 1.52 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.61 (m, 2H, C$^{\gamma}$***H***$_2$), 1.93 (p, ^3J = 7.4 Hz, 2H, COCH$_2$C***H***$_2$CH$_2$CO), 2.28 and 2.36 (m, 4H, 3/5-H piperazine), 2.29 and 2.44 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.30 (m, 2H, COC***H***$_2$(CH$_2$)$_2$CONH), 2.49 (m, 2H, >NCOC***H***$_2$-

cyclopentyl), 2.51 (m, 2H, $CO(CH_2)_2C\boldsymbol{H}_2CONH$), 3.04 (t, ^3J = 5.9 Hz, 2H, $NHCH_2C\boldsymbol{H}_2NH_2$), 3.17 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.34-3.55 (m, 4H, 2/6-H piperazine), 3.44 (t, ^3J = 5.9 Hz, 2H, $NHC\boldsymbol{H}_2CH_2NH_2$), 3.45 and 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.51 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, $\boldsymbol{H}_{ar}$), 7.20 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.43 (m, 1H, $\boldsymbol{H}_{ar}$), 7.47 (m, 1H, $\boldsymbol{H}_{ar}$), 7.54 (m, 1H, $\boldsymbol{H}_{ar}$), 7.87 (m, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 21.0 ($COCH_2\boldsymbol{C}H_2CH_2CO$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 35.4 ($CO\boldsymbol{C}H_2(CH_2)_2CONH$), 36.8 ($CO(CH_2)_2\boldsymbol{C}H_2CONH$), 38.2 ($NH\boldsymbol{C}H_2CH_2NH_2$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 40.9 ($NHCH_2\boldsymbol{C}H_2NH_2$), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 41.9 ($\boldsymbol{C}^{\delta}$), 44.6 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.7 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.8 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.2 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.6 ($\boldsymbol{C}_{ar}H$), 130.0 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 131.6 ($\boldsymbol{C}_{ar}H$), 132.2 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.5 ($\boldsymbol{C}_{ar}H$), 137.0 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.1 ($N\boldsymbol{C}(N)N$), 171.2 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON<$), 174.6 ($\boldsymbol{C}ONH(CH_2)_2N<$), 174.7 ($\boldsymbol{C}ON^{\alpha}H$), 176.4 ($CO(CH_2)_3\boldsymbol{C}ONH$), 176.7 ($\boldsymbol{C}O(CH_2)_3CONH$) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1052 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{56}H_{70}N_{13}O_8$: 1052.5470, found: 1052.5462; RP-HPLC: 100 % (t_R = 10.3 min, k' = 2.9);$C_{56}H_{69}N_{13}O_8$ (1051.54).

(2*S*)-*N*$^{\omega}$-[4-(3-Aminopropyl)aminocarbonylbutanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.49).** Mp 129 °C; ^{1}H NMR (600 MHz, CD_3OD): δ = 1.51 and 1.74 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.50 und 1.66 (m, 4H, 2/5-*H* cyclopentyl), 1.51 und 1.63 (m, 4H, 3/4-*H* cyclopentyl), 1.62 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.83 (p, ^3J = 7.0 Hz, 2H, $CONHCH_2C\boldsymbol{H}_2CH_2NH_2$), 1.92 (p, ^3J = 7.3 Hz, 2H, $COCH_2C\boldsymbol{H}_2CH_2CONH$), 2.17 und 2.24 (m, 4H, 3/5-*H* piperazine), 2.27 (m, 2H, $COC\boldsymbol{H}_2(CH_2)_2CONH$), 2.50 (m, 2H, $CO(CH_2)_2C\boldsymbol{H}_2CONH$), 2.93 (t, ^3J = 7.2 Hz, 2H, $CONH(CH_2)_2C\boldsymbol{H}_2NH_2$), 3.18 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.27 (t, ^3J = 6.6 Hz, 2H, $CONHC\boldsymbol{H}_2(CH_2)_2NH_2$), 3.37 und 3.54 (m, 4H, 2/6-*H* piperazine), 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N$), 3.77 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.33 (br, 1H, 11-H dibenzazepine-11-yl), 7.12 (m, 1H, $\boldsymbol{H}_{ar}$), 7.13 (m, 1H, $\boldsymbol{H}_{ar}$), 7.20 (m, 1H, $\boldsymbol{H}_{ar}$), 7.31 (m, 2H, Ph), 7.34 (m, 1H, $\boldsymbol{H}_{ar}$), 7.37 (m, 8H, Ph), 7.39 (m, 1H, $\boldsymbol{H}_{ar}$), 7.43 (m, 1H, $\boldsymbol{H}_{ar}$), 7.51 (m, 1H, $\boldsymbol{H}_{ar}$), 7.82 (m, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 21.3 ($NCOCH_2\boldsymbol{C}H_2CH_2CONH$), 24.5 und 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 28.8 ($CONHCH_2\boldsymbol{C}H_2CH_2NH_2$), 30.0 ($\boldsymbol{C}^{\beta}$), 35.5 ($NCO\boldsymbol{C}H_2(CH_2)_2CONH$), 36.8 ($NCO(CH_2)_2\boldsymbol{C}H_2CONH$), 38.2 ($CONH\boldsymbol{C}H_2(CH_2)_2NH_2$), 38.5 ($NH\boldsymbol{C}H_2CH_2N$), 39.2 und 39.3 (C-2/5 cyclopentyl), 39.4 und 39.7 ($NCO\boldsymbol{C}H_2$-cyclopentyl), 40.9 ($CONH(CH_2)_2\boldsymbol{C}H_2NH_2$), 41.2 ($NHCH_2\boldsymbol{C}H_2N$), 41.9 ($\boldsymbol{C}^{\delta}$), 42.4 und 47.3 (C-2/6 piperazine), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 52.2 und 52.6 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.2 ($\boldsymbol{C}_{ar}H$), 129.6 ($\boldsymbol{C}_{ar}H$), 130.1 ($\boldsymbol{C}_{ar}$), 130.2 (Ph), 131.2 ($\boldsymbol{C}_{ar}H$), 131.8 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.2 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.1

(N***C***(N)N), 171.8 (***C***=O lactam), 172.7 (***C***ON$^{\alpha}$H), 174.5 (***C***ONHC$_2$H$_4$N), 174.7 (***C***ON), 175.9 (N***C***O(CH$_2$)$_3$CONH), 176.6 (NCO(CH$_2$)$_3$***C***ONH) ppm; IR (neat): υ´ = 3277, 2948, 1648, 1442, 1192, 1130 cm^{-1}; MS (ES, +p) *m/z* (%): 1066 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd for $C_{57}H_{72}N_{13}O_8$: 1066.5621, found: 1066.5642; RP-HPLC: 100 % (t_R = 14.8 min, *k´* = 4.5); anal. calcd. for $C_{57}H_{71}N_{13}O_8 \cdot 1.75\ H_2O \cdot 3.25$ TFA: C 51.95, H 5.32, N 12.40 %, found: C 51.86, H 5.10, N 12.44 %; $C_{57}H_{71}N_{13}O_8$ (1065.55).

(2*S*)-*N*$^{\omega}$-{4-[2-(2-Aminoethyl)aminoethyl]aminocarbonylbutanoyl}-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.50).** Mp 108-110 °C; $[\alpha]_D^{20}$= –10.4 cm^3 g^{-1} dm^{-1} (c = 0.52 in CH$_3$CN/H$_2$O 9:1); ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC, HMBC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 1.93 (p, ^3J = 7.4 Hz, 2H, COCH$_2$C***H***$_2$CH$_2$CO), 2.19 and 2.26 (m, 4H, 3/5-H piperazine), 2.28 and 2.42 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.31 (t, ^3J = 7.4 Hz, 2H, COC***H***$_2$(CH$_2$)$_2$CONH), 2.48 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.50 (t, ^3J = 7.4 Hz, 2H, CO(CH$_2$)$_2$C***H***$_2$CONH), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.22 (t, ^3J = 5.7 Hz, 2H, NHCH$_2$C***H***$_2$NH), 3.33 (t, ^3J = 6.5 Hz, 2H, NHCH$_2$C***H***$_2$NH$_2$), 3.36-3.50 (m, 4H, 2/6-H piperazine), 3.40 (t, ^3J = 6.5 Hz, 2H, NHC***H***$_2$CH$_2$NH$_2$), 3.45 and 3.54 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.51 (t, ^3J = 5.7 Hz, 2H, NHC***H***$_2$CH$_2$NH), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.37 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.40 (m, 1H, ***H***$_{ar}$), 7.44 (m, 1H, ***H***$_{ar}$), 7.51 (m, 1H, ***H***$_{ar}$), 7.83 (d, ^3J = 7.7 Hz, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 21.0 (COCH$_2$***C***H$_2$CH$_2$CO), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 35.3 (CO***C***H$_2$(CH$_2$)$_2$CONH), 36.8 (CO(CH$_2$)$_2$***C***H$_2$CONH), 36.9 (NHCH$_2$***C***H$_2$NH$_2$), 37.1 (CONH***C***H$_2$CH$_2$NH), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.1 (NHCH$_2$***C***H$_2$N<), 42.0 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.7 (NH***C***H$_2$CH$_2$NH$_2$), 45.8 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 49.5 (CONHCH$_2$***C***H$_2$NH), 52.2 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.2 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 130.2 (Ph), 131.3 (***C***$_{ar}$H), 131.9 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.2 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.0 (N***C***(N)N), 171.7 (***C***=O lactam), 172.8 (***C***ON<), 174.6 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H), 176.6 (CO(CH$_2$)$_3$***C***ONH), 176.7 (***C***O(CH$_2$)$_3$CONH) ppm; MS (ES, +p) *m/z* (%): 549 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{58}H_{75}N_{14}O_8$: 1095.5892, found: 1095.5912; RP-HPLC: 99 % (t_R = 13.0 min, *k´* = 3.8); $C_{58}H_{74}N_{14}O_8$ (1094.58).

(2*R*)-*N*$^{\omega}$-{4-[2-(2-Aminoethyl)aminoethyl]aminocarbonylbutanoyl}-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*R*)-3.50). Mp 108-110 °C; $[\alpha]_D^{20}$= +2.5 cm^3 g^{-1} dm^{-1} (c = 0.63 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, CD_3OD, HSQC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 1.93 (p, ^3J = 7.4 Hz, 2H, $COCH_2C\boldsymbol{H}_2CH_2CO$), 2.19 and 2.26 (m, 4H, 3/5-H piperazine), 2.28 and 2.42 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.31 (t, ^3J = 7.4 Hz, 2H, $COC\boldsymbol{H}_2(CH_2)_2CONH$), 2.48 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.50 (t, ^3J = 7.4 Hz, 2H, $CO(CH_2)_2C\boldsymbol{H}_2CONH$), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.22 (t, ^3J = 5.7 Hz, 2H, $NHCH_2C\boldsymbol{H}_2NH$), 3.33 (t, ^3J = 6.5 Hz, 2H, $NHCH_2C\boldsymbol{H}_2NH_2$), 3.36-3.50 (m, 4H, 2/6-H piperazine), 3.40 (t, ^3J = 6.5 Hz, 2H, $NHC\boldsymbol{H}_2CH_2NH_2$), 3.45 and 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.51 (t, ^3J = 5.7 Hz, 2H, $NHC\boldsymbol{H}_2CH_2NH$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.48 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.46 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.86 (d, ^3J = 7.7 Hz, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 21.0 ($COCH_2\boldsymbol{C}H_2CH_2CO$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 35.3 ($CO\boldsymbol{C}H_2(CH_2)_2CONH$), 36.8 ($CO(CH_2)_2\boldsymbol{C}H_2CONH$), 36.9 ($NHCH_2\boldsymbol{C}H_2NH_2$), 37.1 ($CONH\boldsymbol{C}H_2CH_2NH$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.1 ($NHCH_2\boldsymbol{C}H_2N<$), 42.0 (***C***$^{\delta}$), 44.7 (***C***H$_2CON^{\alpha}$H), 45.7 ($NH\boldsymbol{C}H_2CH_2NH_2$), 45.8 (C-1 cyclopentyl), 46.8 (C-2/6 piperazine), 49.5 ($CONHCH_2\boldsymbol{C}H_2NH$), 52.2 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 129.9 (***C***$_{ar}$H), 130.2 (Ph), 130.4 (***C***$_{ar}$H), 131.5 (***C***$_{ar}$H), 132.1 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.0 (NC(N)N), 171.3 (***C***=O lactam), 172.8 (***C***ON<), 174.6 (***C***$ONH(CH_2)_2N<$), 174.7 (***C***ON$^{\alpha}$H), 176.6 ($\boldsymbol{C}O(CH_2)_3\boldsymbol{C}ONH$) ppm; MS (ES, +p) *m/z* (%): 549 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{58}H_{75}N_{14}O_8$: 1095.5892, found: 1095.5912; RP-HPLC: 97 % (t_R = 13.6 min, *k´* = 4.1), gradient: 0 to 20 min: A/B 10/90 to 60/40, 20 to 21 min: 95/5, 21 to 29 min: 95/5; $C_{58}H_{74}N_{14}O_8$ (1094.58).

(2*S*)-*N*$^{\omega}$-(18-Amino-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-3.51). ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.52 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.52 and 1.69 (m, 4H, 2/5-H cyclopentyl), 1.52 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.63 (m, 2H, C$^{\gamma}$***H***$_2$), 1.74 (m, 2H, $NHCH_2C\boldsymbol{H}_2CH_2O$), 1.90 (m, 2H, $H_2NCH_2C\boldsymbol{H}_2CH_2O$), 2.26 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.28 and 2.41 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.55 and 2.73 (m, 4H, $=NCO(C\boldsymbol{H}_2)_2CONH$), 3.08 (m, 2H, $H_2NC\boldsymbol{H}_2(CH_2)_2O$), 3.16 (m, 2H, C$^{\delta}$***H***$_2$), 3.23 (m, 2H, $NHC\boldsymbol{H}_2(CH_2)_2O$), 3.37-3.51 (m, 4H, 2/6-H piperazine), 3.44 and 3.56 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.49 (m, 2H, $NH(CH_2)_2C\boldsymbol{H}_2O$), 3.56 and 3.61 (m, 8H, $(O(C\boldsymbol{H}_2)_2O)_2$), 3.64 (m, 2H, $H_2N(CH_2)_2C\boldsymbol{H}_2O$), 3.78 (m, 2H,

$NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.53 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 1H, $\boldsymbol{H}_{ar}$), 7.17 (m, 1H, $\boldsymbol{H}_{ar}$), 7.20 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.43 (m, 1H, $\boldsymbol{H}_{ar}$), 7.47 (m, 1H, $\boldsymbol{H}_{ar}$), 7.54 (m, 1H, $\boldsymbol{H}_{ar}$), 7.88 (m, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 28.0 ($NHCH_2\boldsymbol{C}H_2CH_2O$), 30.0 ($\boldsymbol{C}^{\beta}$), 30.4 ($H_2NCH_2\boldsymbol{C}H_2CH_2O$), 30.5 (=$NCO\boldsymbol{C}H_2CH_2CO$), 32.9 (=$NCOCH_2\boldsymbol{C}H_2CO$), 37.8 ($NH\boldsymbol{C}H_2(CH_2)_2O$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 40.1 ($H_2N\boldsymbol{C}H_2(CH_2)_2O$), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 41.9 ($\boldsymbol{C}^{\delta}$), 44.6 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.7 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.8 ($\boldsymbol{C}^{\alpha}$), 69.6 ($NH(CH_2)_2\boldsymbol{C}H_2O$), 70.4 ($H_2N(CH_2)_2\boldsymbol{C}H_2O$), 71.0 and 71.4 (($O(\boldsymbol{C}H_2)_2O)_2$), 75.9 (C-11 dibenzazepine-11-yl), 123.1 ($\boldsymbol{C}_{ar}H$), 124.2 (Ph), 126.2 ($\boldsymbol{C}_{ar}H$), 128.2 ($\boldsymbol{C}_{ar}H$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.0 ($\boldsymbol{C}_{ar}$), 130.2 (Ph), 130.5 ($\boldsymbol{C}_{ar}H$), 131.6 ($\boldsymbol{C}_{ar}H$), 132.2 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.5 ($\boldsymbol{C}_{ar}H$), 137.0 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.0 ($N\boldsymbol{C}(N)N$), 171.1 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON^{\alpha}H$), 173.8 (=$NCO(CH_2)_2\boldsymbol{C}O$), 174.5 ($\boldsymbol{C}ONH(CH_2)_2N<$), 174.7 ($\boldsymbol{C}ON<$), 176.4 (=$N\boldsymbol{C}O(CH_2)_2CO$) ppm; MS (ES, +p) *m/z* (%): 1199 (4) $[M+H]^+$, 600 (100) $[M+2H]^{2+}$; HRMS (LSI-MS): *m/z* $[M+2H]^{2+}$ calcd. for $C_{63}H_{84}N_{13}O_{11}$: 599.8246, found: 599.8261; HRMS (FAB): *m/z* $[M+H]^+$ calcd. for $C_{63}H_{84}N_{13}O_{11}$: 1198.6413, found: 1198.6423; RP-HPLC: 99 % (t_R = 15.1 min, k' = 4.6); $C_{63}H_{83}N_{13}O_{11}$ (1197.63).

(2*S*)-N^{ω}-[(2-Aminoethyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-3.52). ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.74 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.59 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.21 and 2.30 (m, 4H, 3/5-H piperazine), 2.28 and 2.43 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 2H, >$NCOC\boldsymbol{H}_2$-cyclopentyl), 3.08 (t, ^3J = 5.7 Hz, 2H, $NHCH_2C\boldsymbol{H}_2NH_2$), 3.15 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.36-3.53 (m, 4H, 2/6-H piperazine), 3.40-3.58 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.48 (m, 2H, $NHC\boldsymbol{H}_2CH_2NH_2$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.41 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, $\boldsymbol{H}_{ar}$), 7.19 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.45 (m, 1H, $\boldsymbol{H}_{ar}$), 7.52 (m, 1H, $\boldsymbol{H}_{ar}$), 7.85 (d, ^3J = 7.7 Hz, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.6 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 38.5 ($NH\boldsymbol{C}H_2CH_2NH_2$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 40.8 ($NHCH_2\boldsymbol{C}H_2NH_2$), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 41.9 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.8 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.2 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.5 ($\boldsymbol{C}_{ar}H$), 129.8 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 130.3 ($\boldsymbol{C}_{ar}$), 131.4 ($\boldsymbol{C}_{ar}H$), 132.0 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.3 ($\boldsymbol{C}_{ar}H$), 137.0 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.7 ($N\boldsymbol{C}(N)N$), 156.5 (=$N\boldsymbol{C}ONH$), 171.5 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON<$), 174.6 ($\boldsymbol{C}ONH(CH_2)_2N<$), 174.7 ($\boldsymbol{C}ON^{\alpha}H$) ppm; MS (ES, +p) *m/z* (%): 492 (100) $[M+2H]^{2+}$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for

$C_{52}H_{64}N_{13}O_7$: 982.5052, found: 982.5071; RP-HPLC: 100 % (t_R = 14.3 min, *k´* = 4.3); $C_{52}H_{63}N_{13}O_7$ (981.50).

(2*S*)-*N*$^{\omega}$-[(3-Aminopropyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.53).** Mp 146 °C; ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 1.86 (p, ^3J = 7.4 Hz, 2H, NHCH$_2$C***H***$_2$CH$_2$NH$_2$), 2.24 and 2.31 (m, 4H, 3/5-H piperazine), 2.28 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.96 (t, ^3J = 7.4 Hz, 2H, NH(CH$_2$)$_2$C***H***$_2$NH$_2$), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.29 (m, 2H, NHC***H***$_2$(CH$_2$)$_2$NH$_2$), 3.39-3.58 (m, 4H, 2/6-H piperazine), 3.46 and 3.54 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.44 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.85 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 28.7 (NHCH$_2$***C***H$_2$CH$_2$NH$_2$), 30.0 (***C***$^{\beta}$), 37.6 (NH***C***H$_2$(CH$_2$)$_2$NH$_2$), 38.3 (NH(CH$_2$)$_2$***C***H$_2$NH$_2$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.2 (NHCH$_2$***C***H$_2$N<), 41.8 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.5 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 130.3 (***C***$_{ar}$), 131.4 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.7 (N***C***(N)N), 156.1 (=N***C***ONH), 171.4 (***C***=O lactam), 172.8 (***C***ON<), 174.6 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; IR (neat): υ´ = 3252, 2949, 1650, 1443, 1180, 1129 cm^{-1}; MS (LSI, MeOH/gly) *m/z* (%): 997 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{53}H_{66}N_{13}O_7$: 996.5208, found: 996.5200; RP-HPLC: 98 % (t_R = 15.0 min, *k´* = 4.5); anal. calcd. for $C_{53}H_{65}N_{13}O_7 \cdot 1.75\ H_2O \cdot 2.75$ TFA: C 52.38, H 5.37, N 13.57 %, found: C 52.36, H 5.32, N 13.56 %; $C_{53}H_{65}N_{13}O_7$ (995.51).

(2*S*)-*N*$^{\omega}$-[(4-Aminobutyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.54).** Mp 146 °C; $[\alpha]_D^{20}$= –1.7 cm^3 g^{-1} dm^{-1} (c = 0.98 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.51 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, NH(CH$_2$)$_2$C***H***$_2$CH$_2$NH$_2$), 1.66 (m, 2H, C$^{\gamma}$***H***$_2$), 1.66 (m, 2H, NHCH$_2$C***H***$_2$(CH$_2$)$_2$NH$_2$), 2.28 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.26 and 2.34 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.93 (t, ^3J = 7.5 Hz, 2H, NH(CH$_2$)$_3$C***H***$_2$NH$_2$), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.21 (t, ^3J = 6.6 Hz, 2H, NHC***H***$_2$(CH$_2$)$_3$NH$_2$), 3.37-3.58 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.47

(br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.86 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$ + NH$(CH_2)_2$***C***$H_2CH_2NH_2$), 27.4 (NHCH$_2$***C***$H_2(CH_2)_2NH_2$), 30.0 (***C***$^{\beta}$), 38.4 (NH***C***H_2CH_2N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H_2-cyclopentyl), 39.9 (NH***C***$H_2(CH_2)_3NH_2$), 39.9 (NH$(CH_2)_3$***C***H_2NH_2), 41.2 (NHCH$_2$***C***H_2N<), 41.8 (***C***$^{\delta}$), 44.6 (***C***$H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.8 (C-2/6 piperazine), 52.3 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 129.9 (***C***$_{ar}$H), 130.2 (Ph), 130.4 (***C***$_{ar}$), 131.5 (***C***$_{ar}$H), 132.1 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.8 (N***C***(N)N), 155.9 (=N***C***ONH), 171.3 (***C***=O lactam), 172.7 (***C***ON<), 174.6 (***C***ONH$(CH_2)_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; IR (neat): υ´ = 3284, 2958, 1652, 1445, 1175, 1131 cm^{-1}; MS (LSI, MeOH/gly) *m/z* (%): 1011 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{54}H_{68}N_{13}O_7$: 1010.5365, found: 1010.5361; RP-HPLC: 98 % (t_R = 15.0 min, *k´* = 4.5); anal. calcd. for $C_{54}H_{67}N_{13}O_7 \cdot 2\ H_2O \cdot 2.5$ TFA: C 53.21, H 5.60, N 13.67 %, found: C 53.24, H 5.87, N 13.77 %; $C_{54}H_{67}N_{13}O_7$ (1009.53).

(2*S*)-*N*$^{\omega}$-[(6-Aminohexyl)aminocarbonyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.55).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.37 (m, 4H, NH$(CH_2)_2$(C***H***$_2)_2(CH_2)_2NH_2$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.52 (m, 2H, NHCH$_2$C***H***$_2(CH_2)_4NH_2$), 1.63 (m, 2H, C$^{\gamma}$***H***$_2$), 1.64 (m, 2H, NH$(CH_2)_4$C***H***$_2CH_2NH_2$), 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 2.20 and 2.28 (m, 4H, 3/5-H piperazine), 2.27 and 2.42 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.90 (t, ^3J = 7.6 Hz, 2H, NH$(CH_2)_5$C***H***$_2NH_2$), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.17 (m, 2H, NHC***H***$_2(CH_2)_5NH_2$), 3.37-3.51 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2CH_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.38 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.31 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.40 (m, 1H, ***H***$_{ar}$), 7.44 (m, 1H, ***H***$_{ar}$), 7.51 (m, 1H, ***H***$_{ar}$), 7.83 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 27.0 and 27.2 (NH$(CH_2)_2$(***C***$H_2)_2(CH_2)_2NH_2$), 28.4 (NH$(CH_2)_4$***C***$H_2CH_2NH_2$), 30.0 (***C***$^{\beta}$), 30.2 (NHCH$_2$***C***$H_2(CH_2)_4NH_2$), 38.4 (NH***C***H_2CH_2N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H_2-cyclopentyl), 40.5 (NH***C***$H_2(CH_2)_5NH_2$), 40.6 (NH$(CH_2)_5$***C***H_2NH_2), 41.2 (NHCH$_2$***C***H_2N<), 41.8 (***C***$^{\delta}$), 44.6 (***C***$H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 47.1 (C-2/6 piperazine), 52.3 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.3 (***C***$_{ar}$H), 129.7 (***C***$_{ar}$H), 130.2 (Ph), 130.2 (***C***$_{ar}$), 131.3 (***C***$_{ar}$H), 131.9 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.6 (=N***C***ONH), 155.8 (N***C***(N)N), 171.6 (***C***=O lactam), 172.7 (***C***ON<), 174.5 (***C***ONH$(CH_2)_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (LSI, MeOH/gly) *m/z* (%):

1039 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{56}H_{72}N_{13}O_7$: 1038.5678, found: 1038.5667; RP-HPLC: 93 % (t_R = 15.5 min, *k´* = 4.7); $C_{56}H_{72}N_{13}O_7$ (1037.56).

(2*S*)-*N*$^{\omega}$-(6-Aminohexanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.56).** $[\alpha]_D^{20}$= +12.0 cm^3 g^{-1} dm^{-1} (c = 0.49 in CH_3CN/H_2O 9:1); ^{1}H NMR (600 MHz, DMSO-d_6, HSQC): δ = 1.31 (m, 2H, $CO(CH_2)_2C\boldsymbol{H}_2(CH_2)_2NH_2$), 1.49 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.45 and 1.64 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.39-1.56 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 2H, $C\boldsymbol{H}_2CH_2NH_2$), 1.54 (m, 2H, $COCH_2C\boldsymbol{H}_2$), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.08 and 2.10 (m, 4H, 3/5-H piperazine), 2.21 and 2.30 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.41 (t, ^3J = 7.3 Hz, 2H, $C(O)C\boldsymbol{H}_2CH_2$), 2.41 and 2.49 (m, 2H, $NCOC\boldsymbol{H}_2$-cyclopentyl), 2.77 (m, 2H, $C\boldsymbol{H}_2NH_2$), 3.18 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.31-3.33 (m, 4H, 2/6-H piperazine), 3.31 and 3.38 (m, 2H, $NHC\boldsymbol{H}_2CH_2N$), 3.60 (t, ^3J = 6.0 Hz, 2H, $NHCH_2C\boldsymbol{H}_2N$), 4.16 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.32 (br, 1H, 11-H dibenzazepine-11-yl), 7.06 (m, 1H, $\boldsymbol{H}_{ar}$), 7.10 (m, 1H, $\boldsymbol{H}_{ar}$), 7.22 (m, 2H, Ph), 7.25 (m, 1H, $\boldsymbol{H}_{ar}$), 7.33 (m, 1H, $\boldsymbol{H}_{ar}$), 7.37 (m, 8H, Ph), 7.39 (m, 1H, $\boldsymbol{H}_{ar}$), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.49 (m, 1H, $\boldsymbol{H}_{ar}$), 7.75 (m, 1H, $\boldsymbol{H}_{ar}$), 7.84 (br, 3H, $N\boldsymbol{H}_3^+$), 7.99 (d, ^3J = 8.0 Hz, 1H, $N^{\alpha}\boldsymbol{H}$), 8.25 (t, ^3J = 5.8 Hz, 1H, $CON\boldsymbol{H}C_2H_4N$), 8.61 and 9.15 (br, 2H, $N^{\omega}\boldsymbol{H}_2$), 9.47 (t, ^3J = 5.4 Hz, 1H, $N^{\delta}\boldsymbol{H}$), 10.36 (s, 1H, C(O)N***H*** lactam), 12.30 (s, 1H, $N^{\omega'}\boldsymbol{H}$) ppm; ^{13}C NMR (150 MHz, DMSO-d_6, HSQC): δ = 23.2 ($COCH_2\boldsymbol{C}H_3$), 23.2 (C-3/4 cyclopentyl), 24.2 ($\boldsymbol{C}^{\gamma}$), 25.2 ($\boldsymbol{C}H_2(CH_2)_2NH_2$), 26.6 ($\boldsymbol{C}H_2CH_2NH_2$), 28.7 ($\boldsymbol{C}^{\beta}$), 35.9 ($C(O)\boldsymbol{C}H_2$), 36.1 ($NH\boldsymbol{C}H_2CH_2N$), 37.2 (C-2/5 cyclopentyl), 38.5 ($NCO\boldsymbol{C}H_2$-cyclopentyl), 38.5 ($\boldsymbol{C}H_2NH_2$), 39.6 ($NHCH_2\boldsymbol{C}H_2N$), 40.5 ($\boldsymbol{C}^{\delta}$), 40.5 and 45.3 (C-2/6 piperazine), 42.8 ($\boldsymbol{C}H_2CON^{\alpha}H$), 44.0 (C-1 cyclopentyl), 50.6 and 51.2 (C-3/5 piperazine), 51.8 ($\boldsymbol{C}^{\alpha}$), 73.6 (C-11 dibenzazepine-11-yl), 121.3 ($\boldsymbol{C}_{ar}H$), 122.6 (Ph), 123.7 ($C_{ar}H$), 126.6 (Ph), 127.8 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 128.4 ($\boldsymbol{C}_{ar}H$), 128.9 (Ph), 129.9 ($\boldsymbol{C}_{ar}H$), 130.5 ($\boldsymbol{C}_{ar}H$), 131.3 ($\boldsymbol{C}_{ar}H$), 131.6 ($\boldsymbol{C}_{ar}$), 136.1 ($\boldsymbol{C}_{ar}$), 136.5 ($\boldsymbol{C}_{ar}$), 141.5 ($\boldsymbol{C}_{ar}$), 152.5 ($N(\boldsymbol{C}ONPh)_2$), 153.1 ($N\boldsymbol{C}(N)N$), 167.9 (***C***=O lactam), 169.9 (***C***ON), 171.2 ($\boldsymbol{C}ON^{\alpha}H$), 171.9 ($\boldsymbol{C}ONHC_2H_4N$), 175.3 ($\boldsymbol{C}O(CH_2)_5$) ppm; IR (neat): υ´ = 3298, 2954, 1665, 1447, 1179, 1130 cm^{-1}; MS (ES, +p) *m/z* (%): 1010 (32) $[M+H]^+$, 803 (70) $[MH–207]^+$; RP-HPLC: 100 % (t_R = 15.2 min, *k´* = 4.6); anal. calcd. for $C_{55}H_{68}N_{12}O_7 \cdot 5\ H_2O \cdot 5$ HCl: C 51.54, H 6.53, N 13.12 %, found: C 51.23, H 6.53, N 13.00%; $C_{55}H_{68}N_{12}O_7$ (1009.20).

(2*S*)-*N*$^{\omega}$-(8-Amino-3,6-dioxaoctanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*S*)-**3.57).** Mp 142-145 °C; ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.77 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.63 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.20 and 2.29 (m, 4H, 3/5-H piperazine), 2.27 and 2.43 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.47 (m, 2H, $>NCOC\boldsymbol{H}_2$-cyclopentyl), 3.12 (t, ^3J = 5.0 Hz, 2H, $OCH_2C\boldsymbol{H}_2NH_2$), 3.20 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.37-3.51 (m, 4H, 2/6-H piperazine), 3.45 and 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.70 (m, 2H, $OC\boldsymbol{H}_2CH_2NH_2$), 3.72 and 3.78 (m, 4H, $O(C\boldsymbol{H}_2)_2O$),

3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.25 (s, 2H, =NCOC***H***$_2$O), 4.40 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.84 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.6 (OCH$_2$***C***H$_2$NH$_2$), 41.1 (NHCH$_2$***C***H$_2$N<), 42.1 (***C***$^{\delta}$), 44.6 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 47.1 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 67.9 (O***C***H$_2$CH$_2$NH$_2$), 71.1 (=NCO***C***H$_2$O), 71.3 and 72.1 (O(***C***H$_2$)$_2$O), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.4 (***C***$_{ar}$H), 129.7 (***C***$_{ar}$H), 130.2 (Ph), 130.2 (***C***$_{ar}$), 131.3 (***C***$_{ar}$H), 131.9 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.8 (N***C***(N)N), 171.6 (***C***=O lactam), 172.8 (***C***ON<), 174.0 (=N***C***OCH$_2$O), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1042 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{55}H_{69}N_{12}O_9$: 1041.5310, found: 1041.5272; RP-HPLC: 93 % (t_R = 15.2 min, *k´* = 4.6); $C_{55}H_{68}N_{12}O_9$ (1040.52).

(2*S*)-*N*$^{\omega}$-(15-Amino-4,7,10,13-tetraoxapentadecanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.58).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.52 and 1.74 (m, 4H, 2/5-H cyclopentyl), 1.50 and 1.69 (m, 2H, C$^{\beta}$***H***$_2$), 1.60 (m, 2H, C$^{\gamma}$***H***$_2$), 2.18 and 2.25 (m, 4H, 3/5-H piperazine), 2.27 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.47 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.72 (t, ^3J = 5.9 Hz, 2H, =NCOC***H***$_2$CH$_2$O), 3.12 (t, ^3J = 5.0 Hz, 2H, OCH$_2$C***H***$_2$NH$_2$), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.35-3.51 (m, 4H, 2/6-H piperazine), 3.44 and 3.53 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.62 and 3.64 (m, 12H, (O(C***H***$_2$)$_2$O)$_3$), 3.70 (t, ^3J = 5.0 Hz, 2H, OC***H***$_2$CH$_2$NH$_2$), 3.78 (t, ^3J = 5.9 Hz, 2H, =NCOCH$_2$C***H***$_2$O), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.36 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.40 (m, 1H, ***H***$_{ar}$), 7.44 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.83 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 29.9 (***C***$^{\beta}$), 38.4 (NH***C***H$_2$CH$_2$N<), 38.4 (=NCO***C***H$_2$CH$_2$O), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.6 (OCH$_2$***C***H$_2$NH$_2$), 41.1 (NHCH$_2$***C***H$_2$N<), 42.0 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 66.9 (=NCOCH$_2$***C***H$_2$O), 67.8 (O***C***H$_2$CH$_2$NH$_2$), 71.1 and 71.3 and 71.5 ((O(***C***H$_2$)$_2$O)$_3$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.2 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 130.2 (Ph), 131.3 (***C***$_{ar}$H), 131.9 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.2 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.0 (N***C***(N)N), 171.7 (***C***=O lactam), 172.7 (***C***ON<), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H), 175.4 (=N***C***O(CH$_2$)$_2$O) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1144 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd for : , found: ; RP-HPLC: 93 % (t_R = 15.4 min, *k´* = 4.7); $C_{60}H_{78}N_{12}O_{11}$ (1142.59).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoyl}argininamide ((*S*)-**3.59).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.51 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.51 and 1.72 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.81 (m, 2H, >NCOCH$_2C\boldsymbol{H}_2CH_2$CO), 2.23 (t, ^{3}J = 7.1 Hz, 2H, >NCO(CH$_2$)$_2$C$\boldsymbol{H}_2$CO), 2.28 and 2.33 (m, 4H, 3/5-H piperazine), 2.34 (m, 2H, >NCOC$\boldsymbol{H}_2$(CH$_2$)$_2$CO), 3.02 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.37 and 3.43 (m, 4H, 2/6-H piperazine), 3.46 and 3.57 (m, 2H, NHC$\boldsymbol{H}_2$CH$_2$N<), 3.77 (m, 2H, NHCH$_2$C$\boldsymbol{H}_2$N<), 4.24 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.48 (br, 1H, 11-H dibenzazepin-11-yl), 7.15 (m, 2H, $\boldsymbol{H}_{ar}$), 7.21 (m, 2H, $\boldsymbol{H}_{ar}$), 7.33 (m, 5H, Ph), 7.38 (m, 5H, Ph), 7.42 (m, 1H, $\boldsymbol{H}_{ar}$), 7.46 (m, 1H, $\boldsymbol{H}_{ar}$), 7.53 (m, 1H, $\boldsymbol{H}_{ar}$), 7.86 (d, ^{3}J = 7.6 Hz, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 22.2 (>NCOCH$_2$C$\boldsymbol{H}_2$CH$_2$CO), 26.2 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 33.1 (>NCO$\boldsymbol{C}$H$_2$(CH$_2$)$_2$CO), 35.7 (>NCO(CH$_2$)$_2\boldsymbol{C}$H$_2$CO), 38.4 (NH$\boldsymbol{C}$H$_2$CH$_2$N<), 41.3 (NHCH$_2\boldsymbol{C}$H$_2$N<), 41.9 ($\boldsymbol{C}^{\delta}$), 46.0 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 54.1 ($\boldsymbol{C}^{\alpha}$), 75.9 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}$H), 124.2 (Ph), 126.2 ($\boldsymbol{C}_{ar}$H), 128.2 ($\boldsymbol{C}_{ar}$H), 129.6 ($\boldsymbol{C}_{ar}$H), 129.9 ($\boldsymbol{C}_{ar}$H), 130.2 (Ph), 130.5 ($\boldsymbol{C}_{ar}$H), 131.5 ($\boldsymbol{C}_{ar}$H), 132.1 ($\boldsymbol{C}_{ar}$H), 132.4 ($\boldsymbol{C}_{ar}$), 133.4 ($\boldsymbol{C}_{ar}$H), 137.0 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 (N($\boldsymbol{C}$ONPh)$_2$), 158.6 (N$\boldsymbol{C}$(N)N), 171.2 ($\boldsymbol{C}$=O lactam), 173.2 ($\boldsymbol{C}$ON<), 174.6 ($\boldsymbol{C}$ONH(CH$_2$)$_2$N<), 175.5 ($\boldsymbol{C}$ON$^{\alpha}$H) ppm; MS (ES, +p) *m/z* (%): 842 (50) [*M*+H]$^{+}$, 422 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^{+}$ calcd. for $C_{45}H_{52}N_{11}O_6$: 842.4102, found: 842.4089; RP-HPLC: 99 % (t_R = 14.3 min, *k´* = 4.3); $C_{45}H_{51}N_{11}O_6$ (841.40).

(2*S*)-N^{ω}-{4-[2-(2-Aminoethyl)aminoethyl]aminocarbonylbutanoyl}-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-{5-oxo-5-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]pentanoyl}argininamide ((*S*)-**3.60).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.52 and 1.74 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.59 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.81 (m, 2H, >NCOCH$_2C\boldsymbol{H}_2CH_2$CO), 1.93 (p, ^{3}J = 7.4 Hz, 2H, =NCOCH$_2$C$\boldsymbol{H}_2$CH$_2$CO), 2.23 (t, ^{3}J = 7.0 Hz, 2H, >NCO(CH$_2$)$_2$C$\boldsymbol{H}_2$CO), 2.23 and 2.29 (m, 4H, 3/5-H piperazine), 2.29 and 2.35 (m, 2H, >NCOC$\boldsymbol{H}_2$(CH$_2$)$_2$CO), 2.31 and 2.51 (m, 2H, t, ^{3}J = 7.4 Hz, 2H, =NCOC$\boldsymbol{H}_2$CH$_2$C$\boldsymbol{H}_2$CO), 3.16 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.22 (t, ^{3}J = 5.7 Hz, 2H, NHCH$_2$C$\boldsymbol{H}_2$NH), 3.33 (t, ^{3}J = 6.0 Hz, 2H, NHCH$_2$C$\boldsymbol{H}_2$NH$_2$), 3.36 (m, 4H, 2/6-H piperazine), 3.40 (t, ^{3}J = 6.0 Hz, 2H, NHC$\boldsymbol{H}_2$CH$_2$NH$_2$), 3.47 and 3.53 (m, 2H, NHC$\boldsymbol{H}_2$CH$_2$N<), 3.51 (t, ^{3}J = 5.7 Hz, 2H, NHC$\boldsymbol{H}_2$CH$_2$NH), 3.77 (m, 2H, NHCH$_2$C$\boldsymbol{H}_2$N<), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.42 (br, 1H, 11-H dibenzazepin-11-yl), 7.15 (m, 2H, $\boldsymbol{H}_{ar}$), 7.21 (m, 2H, $\boldsymbol{H}_{ar}$), 7.33 (m, 5H, Ph), 7.38 (m, 5H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.45 (m, 1H, $\boldsymbol{H}_{ar}$), 7.53 (m, 1H, $\boldsymbol{H}_{ar}$), 7.86 (d, ^{3}J = 7.7 Hz, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 20.9 (=NCOCH$_2\boldsymbol{C}$H$_2$CH$_2$CONH), 22.2 (>NCOCH$_2\boldsymbol{C}$H$_2$CH$_2$CO), 25.4 ($\boldsymbol{C}^{\gamma}$), 29.9 ($\boldsymbol{C}^{\beta}$), 33.1 (>NCO$\boldsymbol{C}$H$_2$(CH$_2$)$_2$CO), 35.3 and 36.8 (=NCO$\boldsymbol{C}$H$_2$CH$_2\boldsymbol{C}$H$_2$CONH), 35.7 (>NCO(CH$_2$)$_2\boldsymbol{C}$H$_2$CO), 36.9 (NHCH$_2\boldsymbol{C}$H$_2$NH$_2$), 37.1 (CONH$\boldsymbol{C}$H$_2$CH$_2$NH), 38.4 (NH$\boldsymbol{C}$H$_2$CH$_2$N<), 41.3 (NHCH$_2\boldsymbol{C}$H$_2$N<), 41.9 ($\boldsymbol{C}^{\delta}$), 45.7 (NH$\boldsymbol{C}$H$_2$CH$_2$NH$_2$), 46.2 (C-2/6 piperazine), 49.5 (CONHCH$_2\boldsymbol{C}$H$_2$NH), 52.2 and 52.6 (C-3/5 piperazine), 54.0 ($\boldsymbol{C}^{\alpha}$), 75.9 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}$H), 124.2 (Ph), 126.2 ($\boldsymbol{C}_{ar}$H), 128.2 ($\boldsymbol{C}_{ar}$H), 129.4 ($\boldsymbol{C}_{ar}$H), 129.8 ($\boldsymbol{C}_{ar}$H), 130.2 (Ph), 130.3 ($\boldsymbol{C}_{ar}$H), 131.4 ($\boldsymbol{C}_{ar}$H), 132.0 ($\boldsymbol{C}_{ar}$H), 132.4 ($\boldsymbol{C}_{ar}$), 133.3 ($\boldsymbol{C}_{ar}$H), 137.0 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 (N($\boldsymbol{C}$ONPh)$_2$), 155.1

(N***C***(N)N), 171.5 (***C***=O lactam), 173.2 (***C***ON<), 174.5 (***C***ONH$(CH_2)_2$N<), 175.5 (***C***ON$^{\alpha}$H), 176.6 and 176.7 (=N***C***O$(CH_2)_3$***C***ONH) ppm; MS (ES, +p) *m/z* (%): 1041 (10) $[M+H]^+$, 521 (80) $[M+2H]^{2+}$, 348 (100) $[M+3H]^{3+}$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{54}H_{69}N_{14}O_8$: 1041.5423, found: 1041.5410; RP-HPLC: 99 % (t_R = 11.8 min, *k´* = 3.4); $C_{54}H_{68}N_{14}O_8$ (1040.53).

(2*S*)-*N*$^{\alpha}$-[2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginylphenylalaninamide (3.61). Mp 148 °C; ^{1}H NMR (400 MHz, CD_3OD, HSQC): δ = 1.47 (m, 2H, C$^{\gamma}$***H***$_2$), 1.50 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.57 and 1.65 (m, 2H, C$^{\beta}$***H***$_2$ Orn), 2.28 and 2.47 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.30 (m, 4H, 3/5-H piperazine), 2.45 and 2.50 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.87 and 3.17 (m, 2H, C$^{\beta}$***H***$_2$ Phe-amide), 3.09 (t, ^3J = 6.9 Hz, 2H, C$^{\delta}$***H***$_2$), 3.34-3.55 (m, 4H, 2/6-H piperazine), 4.21 (m, 1H, C$^{\alpha}$***H*** Orn), 4.43 (m, 1H, 11-H dibenzazepine-11-yl), 4.58 (m, 1H, C$^{\alpha}$***H*** Phe-amide), 7.15 (m, 4H, Ph), 7.22 (m, 3H), 7.30 (m, 1H, ***H***$_{ar}$), 7.43 (m, 3H), 7.52 (m, 1H, ***H***$_{ar}$), 7.85 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (100 MHz, CD_3OD, HSQC): δ = 23.2 (C-3/4 cyclopentyl), 24.6 (***C***$^{\gamma}$), 28.4 (***C***$^{\beta}$ Orn), 37.2 (***C***$^{\beta}$ Phe-amide), 38.1 and 38.2 (C-2/5 cyclopentyl), 38.4 (>NCO***C***H_2-cyclopentyl), 40.5 (***C***$^{\delta}$), 43.1 (***C***H_2CON$^{\alpha}$H), 44.3 (C-1 cyclopentyl), 45.5 (C-2/6 piperazine), 50.8 and 51.2 (C-3/5 piperazine), 53.0 (***C***$^{\alpha}$ Orn), 54.3 (***C***$^{\alpha}$ Phe-amide), 74.4 (C-11 dibenzazepine-11-yl), 121.6 (***C***$_{ar}$H), 124.8 (***C***$_{ar}$H), 126.3 (Ph), 128.0 (Ph), 128.4 (***C***$_{ar}$H), 129.0 (Ph), 130.0 (***C***$_{ar}$H), 130.6 (***C***$_{ar}$H), 131.0 (***C***$_{ar}$), 132.0 (***C***$_{ar}$H), 135.5 (***C***$_{ar}$), 137.4 (***C***$_{ar}$), 157.2 (N***C***(N)N), 170.1 (***C***=O lactam), 171.5 (***C***ON<), 172.5 (***C***ON$^{\alpha}$H Phe-amide), 173.8 (***C***ON$^{\alpha}$H Orn), 174.7 (***C***ONH_2) ppm; MS (LSI, MeOH/gly) *m/z* (%): 765 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{42}H_{54}N_9O_5$: 764.4248, found: 764.4247; RP-HPLC: 98 % (t_R = 14.9 min, *k´* = 4.5); $C_{42}H_{53}N_9O_5$ (763.42).

(2*S*)-*N*$^{\omega}$-[(3-Aminopropyl)aminocarbonyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginyl-phenylalaninamide (3.62). ^{1}H NMR (400 MHz, CD_3OD, HSQC): δ = 1.50 (m, 2H, C$^{\gamma}$***H***$_2$), 1.50 and 1.65 (m, 2H, C$^{\beta}$***H***$_2$ Orn), 1.50 and 1.71 (m, 4H, 2/5-H cyclopentyl), 1.64 (m, 4H, 3/4-H cyclopentyl), 1.88 (p, ^3J = 7.2 Hz, 2H, $NHCH_2C$***H***$_2CH_2NH_2$), 2.28 and 2.47 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.28 (m, 4H, 3/5-H piperazine), 2.46 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.87 and 3.17 (m, 2H, C$^{\beta}$***H***$_2$ Phe-amide), 2.98 (t, ^3J = 7.5 Hz, 2H, NH$(CH_2)_2$C***H***$_2NH_2$), 3.20 (m, 2H, C$^{\delta}$***H***$_2$), 3.30 (m, 2H, NHC***H***$_2(CH_2)_2NH_2$), 3.35-3.54 (m, 4H, 2/6-H piperazine), 4.19 (m, 1H, C$^{\alpha}$***H*** Orn), 4.40 (m, 1H, 11-H dibenzazepine-11-yl), 4.59 (m, 1H, C$^{\alpha}$***H*** Phe-amide), 7.11-7.26 (m, 7H), 7.30 (m, 1H, ***H***$_{ar}$), 7.35 (m, 1H, ***H***$_{ar}$), 7.43 (m, 2H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.85 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (100 MHz, CD_3OD, HSQC): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.5 (***C***$^{\gamma}$), 28.7 ($NHCH_2$***C***$H_2CH_2NH_2$), 29.6 and 29.7 (***C***$^{\beta}$ Orn), 37.6 (NH***C***$H_2(CH_2)_2NH_2$), 38.3 (NH$(CH_2)_2$***C***H_2NH_2), 38.4 and 38.5 (***C***$^{\beta}$ Phe-amide), 39.4 and 39.5 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H_2-cyclopentyl), 41.8 (***C***$^{\delta}$), 44.4 (***C***H_2CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 54.3

and 54.4 (***C***$^{\alpha}$ Orn), 55.7 (***C***$^{\alpha}$ Phe-amide), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 126.2 (***C***$_{ar}$H), 127.7 (***C***$_{ar}$H), 128.8 (***C***$_{ar}$H), 129.4 (Ph), 129.7 (***C***$_{ar}$H), 130.3 (Ph), 131.4 (***C***$_{ar}$), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 138.8 (***C***$_{ar}$), 155.8 (N***C***(N)N), 156.1 (=N***C***ONH), 171.6 (***C***=O lactam), 172.8 (***C***ON<), 173.9 (***C***ON$^{\alpha}$H Phe-amide), 175.3 (***C***ON$^{\alpha}$H Orn), 176.0 (***C***ONH$_2$) ppm; MS (LSI, MeOH/gly) *m/z* (%): 865 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{46}H_{62}N_{11}O_6$: 864.4885, found: 864.4899; RP-HPLC: 97 % (t_R = 13.1 min, *k´* = 3.9); $C_{46}H_{61}N_{11}O_6$ (863.48).

General procedure for the preparation of the potential radioligands (*S*)-3.63-(*S*)-3.84:[21]

The pertinent amine precursor (1 eq) and NEt_3 (2.5 eq) were dissolved in CH_3CN (1 mL/20 µmol) followed by the addition of the corresponding active ester (1 eq) in CH_3CN (0.5 mL/20 µmol). The reaction was stopped by addition of 10 % aq. TFA (corresponding to 2-3 eq of TFA) after an incubation period of 16 h at rt. Purification with preparative HPLC and lyophilization afforded the products as white fluffy solids in yields from 65 to 90 %.

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-[4-(2-propanoylaminoethyl)aminocarbonylbutanoyl]argininamide ((*S*)-**3.63).** Mp 127 °C; ^{1}H NMR (600 MHz, CD_3OD, HSQC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, $COCH_2CH_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.52 and 1.75 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 1.91 (p, ^3J = 7.3 Hz, 2H, $COCH_2CH_2CH_2CO$), 2.18 and 2.25 (m, 4H, 3/5-H piperazine), 2.19 (quat, ^3J = 7.6 Hz, 2H, $COCH_2CH_3$), 2.24 (t, ^3J = 7.3 Hz, 2H, $COCH_2(CH_2)_2CONH$), 2.25 and 2.42 (m, 2H, $CH_2CON^{\alpha}H$), 2.47 (m, 2H, >NCOCH_2-cyclopentyl), 2.47 (m, 2H, $CO(CH_2)_2CH_2CONH$), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.27 (m, 4H, $CONH(CH_2)_2NH$), 3.33-3.53 (m, 4H, 2/6-H piperazine), 3.46 and 3.54 (m, 2H, $NHCH_2CH_2N$<), 3.78 (m, 2H, $NHCH_2CH_2N$<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.34 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 6H, Ph), 7.44 (m, 1H, ***H***$_{ar}$), 7.51 (m, 1H, ***H***$_{ar}$), 7.83 (d, ^3J = 7.6 Hz, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 10.4 ($COCH_2CH_3$), 21.4 ($COCH_2CH_2CH_2CO$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.2 ($COCH_2CH_3$), 35.6 ($COCH_2(CH_2)_2CONH$), 36.9 ($CO(CH_2)_2CH_2CONH$), 38.5 ($NHCH_2CH_2N$<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.6 (>NCOCH_2-cyclopentyl), 40.0 and 40.2 ($CONH(CH_2)_2NH$), 41.2 ($NHCH_2CH_2N$<), 42.0 (***C***$^{\delta}$), 44.7 ($CH_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 47.1 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.3 (***C***$_{ar}$H), 129.7 (***C***$_{ar}$H), 130.2 (Ph), 131.3 (***C***$_{ar}$H), 131.8 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.2 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.9 (N***C***(N)N), 172.8 (***C***ON<), 174.5 (***C***ONH$(CH_2)_2$N<), 174.7 (***C***ON$^{\alpha}$H), 176.4 (***C***O$(CH_2)_3$*C*ONH), 177.4 (***C***OCH_2CH_3) ppm; MS

(ES, +p) *m/z* (%): 1108 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{59}H_{74}N_{13}O_9$: 1108.5732, found: 1108.5712; RP-HPLC: 95 % (t_R = 17.0 min, k' = 5.3); $C_{59}H_{73}N_{13}O_9$ (1107.57).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-[4-(3-propanoylaminopropyl)aminocarbonylbutanoyl]argininamide ((*S*)-**3.64).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.11 ($COCH_2CH_3$), 1.51 and 1.74 (m, 2H, $C^{\beta}H_2$), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.62 (m, 2H, $C^{\gamma}H_2$), 1.66 (m, 2H, $CONHCH_2CH_2CH_2NH$), 1.92 (p, ^3J = 7.3 Hz, 2H, $COCH_2CH_2CH_2CONH(CH_2)_3NH$), 2.18 and 2.25 (m, 4H, 3/5-H piperazine), 2.20 (m, 2H, $COCH_2CH_3$), 2.27 (m, 2H, $COCH_2(CH_2)_2CONH(CH_2)_3NH$), 2.48 (m, 2H, $CO(CH_2)_2CH_2CONH(CH_2)_3NH$), 3.18 (m, 6H, $CONHCH_2CH_2CH_2NH_3$, $C^{\delta}H_2$), 3.34-3.48 (m, 4H, 2/6-H piperazine), 3.54 (m, 2H, $NHCH_2CH_2N$), 3.78 (m, 2H, $NHCH_2CH_2N$), 4.25 (m, 1H, $C^{\alpha}H_2$), 4.41 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 1H, H_{ar}), 7.15 (m, 1H, H_{ar}), 7.20 (m, 2H, H_{ar}), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, H_{ar}), 7.45 (m, 1H, H_{ar}), 7.52 (m, 1H, H_{ar}), 7.85 (m, 1H, H_{ar}) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 10.5 ($COCH_2CH_3$), 21.4 ($=NCOCH_2CH_2CH_2CONH$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (C^{γ}), 28.8 ($CONHCH_2CH_2CH_2NH$), 30.0 (C^{β}), 30.2 ($COCH_2CH_3$), 35.6 ($=NCOCH_2(CH_2)_2CONH(CH_2)_3NH$), 36.8 ($=NCO(CH_2)_2CH_2CONH(CH_2)_3NH$), 37.7 ($CONHCH_2CH_2CH_2NH$), 38.2 ($CONHCH_2CH_2CH_2NH$), 38.5 ($NHCH_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.5 and 39.7 (>$NCOCH_2$-cyclopentyl), 41.2 ($NHCH_2CH_2N<$), 42.0 (C^{δ}), 44.7 ($CH_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 53.7 (C^{α}), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($C_{ar}H$), 124.2 (Ph), 126.1 ($C_{ar}H$), 128.2 ($C_{ar}H$), 129.3 ($C_{ar}H$), 129.7 ($C_{ar}H$), 130.2 (Ph), 131.4 ($C_{ar}H$), 132.0 ($C_{ar}H$), 132.4 (C_{ar}), 133.3 ($C_{ar}H$), 137.0 (C_{ar}), 137.8 (C_{ar}), 154.5 ($N(CONPh)_2$), 155.0 (NC(N)N), 172.8 ($CON^{\alpha}H$), 174.5 ($CONHC_2H_4N<$), 174.7 ($CON<$), 175.9 ($=NCO(CH_2)_3CONH$), 176.4 ($=NCO(CH_2)_3CONH$), 177.2 ($NHCOCH_2CH_3$) ppm; MS (ES, +p) *m/z* (%): 1122 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd for $C_{60}H_{76}N_{13}O_9$: 1122.5889, found: 1122.5834; RP-HPLC: 99 % (t_R = 14.7 min, k' = 4.4); $C_{60}H_{75}N_{13}O_9$ (1121.58).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-{4-[2-(2-propanoylaminoethyl)aminoethyl]aminocarbonylbutanoyl}-argininamide ((*S*)-**3.65).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.12 (t, ^3J = 7.6 Hz, 3H, $COCH_2CH_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.52 and 1.75 (m, 2H, $C^{\beta}H_2$), 1.60 (m, 2H, $C^{\gamma}H_2$), 1.93 (p, ^3J = 7.4 Hz, 2H, $COCH_2CH_2CH_2CO$), 2.20 and 2.28 (m, 4H, 3/5-H piperazine), 2.25 (m, 2H, $COCH_2CH_3$), 2.26 and 2.43 (m, 2H, $CH_2CON^{\alpha}H$), 2.32 (t, ^3J = 7.4 Hz, 2H, $COCH_2(CH_2)_2CONH$), 2.48 (m, 2H, >$NCOCH_2$-cyclopentyl), 2.51 (m, 2H,

$CO(CH_2)_2C\boldsymbol{H}_2CONH$), 3.16 (m, 4H, 2 x $CONHCH_2C\boldsymbol{H}_2NH$), 3.18 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.35-3.52 (m, 4H, 2/6-H piperazine), 3.45 and 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.46 (m, 4H, $CONHC\boldsymbol{H}_2CH_2NH$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.39 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, $\boldsymbol{H}_{ar}$), 7.20 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.45 (m, 1H, $\boldsymbol{H}_{ar}$), 7.52 (m, 1H, $\boldsymbol{H}_{ar}$), 7.84 (d, ^3J = 7.7 Hz, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 10.0 ($COCH_2\boldsymbol{C}H_3$), 21.0 ($COCH_2\boldsymbol{C}H_2CH_2CO$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 29.8 ($CO\boldsymbol{C}H_2CH_3$), 30.0 ($\boldsymbol{C}^{\beta}$), 35.3 ($CO\boldsymbol{C}H_2(CH_2)_2CONH$), 36.8 ($CO(CH_2)_2\boldsymbol{C}H_2CONH$), 37.3 (2 x $CONH\boldsymbol{C}H_2CH_2NH$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 42.0 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 47.1 (C-2/6 piperazine), 49.3 (2 x $CONHCH_2\boldsymbol{C}H_2NH$), 52.2 and 52.6 (C-3/5 piperazine), 53.8 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.3 ($\boldsymbol{C}_{ar}H$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 131.3 ($\boldsymbol{C}_{ar}H$), 131.9 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.3 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.0 ($N\boldsymbol{C}(N)N$), 171.6 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON<$), 174.6 ($\boldsymbol{C}ONH(CH_2)_2N<$), 174.7 ($\boldsymbol{C}ON^{\alpha}H$), 176.6 ($CO(CH_2)_3\boldsymbol{C}ONH$), 176.7 ($\boldsymbol{C}O(CH_2)_3CONH$), 178.7 ($\boldsymbol{C}OCH_2CH_3$) ppm; MS (ES, +p) *m/z* (%): 576 (65) $[M+2H]^{2+}$, 385 (100) $[M+3H]^{3+}$; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd. for $C_{61}H_{79}N_{14}O_9$: 1151.6149, found: 1151.6144; RP-HPLC: 98 % (t_R = 14.5 min, k' = 4.4); $C_{61}H_{78}N_{14}O_9$ (1150.61).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-(18-propanoylamino-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl)arginin-amide ((*S*)-**3.66).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, $COCH_2C\boldsymbol{H}_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.52 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.58 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.73 (m, 4H, $(NHCH_2(C\boldsymbol{H}_2CH_2O)_2)$), 2.25 and 2.42 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.16-2.28 (m, 4H, 3/5-H piperazine), 2.17 (quat, ^3J = 7.6 Hz, 2H, $COC\boldsymbol{H}_2CH_3$), 2.48 (m, 2H, >$NCOC\boldsymbol{H}_2$-cyclopentyl), 2.55 and 2.69 (m, 4H, $=NCO(C\boldsymbol{H}_2)_2CONH$), 3.16 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.23 (m, 4H, $(NHC\boldsymbol{H}_2(CH_2)_2O)_2$), 3.37-3.51 (m, 4H, 2/6-H piperazine), 3.44 and 3.56 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.50 (m, 4H, $(NH(CH_2)_2C\boldsymbol{H}_2O)_2$), 3.56 and 3.62 (m, 8H, $(O(C\boldsymbol{H}_2)_2O)_2$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.37 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, $\boldsymbol{H}_{ar}$), 7.20 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.44 (m, 1H, $\boldsymbol{H}_{ar}$), 7.51 (m, 1H, $\boldsymbol{H}_{ar}$), 7.83 (m, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 10.6 ($COCH_2\boldsymbol{C}H_3$), 24.6 and 24.7 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 30.3 ($CO\boldsymbol{C}H_2CH_3$), 30.4 ($NHCH_2\boldsymbol{C}H_2CH_2O$), 30.4 ($=NCO\boldsymbol{C}H_2CH_2CO$), 33.0 ($=NCOCH_2\boldsymbol{C}H_2CO$), 37.9 ($NH\boldsymbol{C}H_2(CH_2)_2O$), 38.5 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 42.1 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 54.8 ($CH_3CH_2CONH\boldsymbol{C}H_2(CH_2)_2O$), 69.9 ($(NH(CH_2)_2\boldsymbol{C}H_2O)_2$), 71.2 and 71.5 ($(O(\boldsymbol{C}H_2)_2O)_2$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.2 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.2 ($\boldsymbol{C}_{ar}H$), 129.2 ($\boldsymbol{C}_{ar}$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 131.3 ($\boldsymbol{C}_{ar}H$), 131.9 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.2 ($\boldsymbol{C}_{ar}H$), 136.9

(***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.9 (N***C***(N)N), 172.8 (***C***ON$^{\alpha}$H), 173.7 (=NCO(CH$_2$)$_2$***C***O), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON<), 176.3 (=N***C***O(CH$_2$)$_2$CO), 177.0 (***C***OCH$_2$CH$_3$) ppm; MS (ES, +p) *m/z* (%): 628 (100) [*M*+2H]$^{2+}$; HRMS (ESI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{66}H_{88}N_{13}O_{12}$: 1254.6670, found: 1254.6670; RP-HPLC: 99 % (t_R = 17.7 min, *k´* = 5.6); $C_{66}H_{87}N_{13}O_{12}$ (1253.66).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-[(2-propanoylaminoethyl)aminocarbonyl]argininamide ((*S*)-3.67). ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC, HMBC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, COCH$_2$C***H***$_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.73 (m, 2H, C$^{\beta}$***H***$_2$), 1.57 (m, 2H, C$^{\gamma}$***H***$_2$), 2.19 (quat, ^3J = 7.6 Hz, 2H, COC***H***$_2$CH$_3$), 2.22 and 2.29 (m, 4H, 3/5-H piperazine), 2.28 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.48 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.27 (m, 2H, NHC***H***$_2$CH$_2$NHCOCH$_2$CH$_3$), 3.29 (m, 2H, NHCH$_2$C***H***$_2$NHCOCH$_2$CH$_3$), 3.44 (br, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.40 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.85 (d, ^3J = 7.5 Hz, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 10.4 (COCH$_2$***C***H$_3$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.6 (***C***$^{\gamma}$), 30.1 (***C***$^{\beta}$), 30.2 (CO***C***H$_2$CH$_3$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 39.9 (NHCH$_2$***C***H$_2$COCH$_2$CH$_3$), 40.7 (NH***C***H$_2$CH$_2$NHCOCH$_2$CH$_3$), 41.2 (NHCH$_2$***C***H$_2$N<), 41.9 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.5 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 130.3 (***C***$_{ar}$), 131.4 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.6 (=N***C***ONH), 155.7 (N***C***(N)N), 171.6 (***C***=O lactam), 172.8 (***C***ON<), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H), 177.5 (***C***OCH$_2$CH$_3$) ppm; MS (ES, +p) *m/z* (%): 1039 (45) [*M*+H]$^+$, 520 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{55}H_{68}N_{13}O_8$: 1038.5314, found: 1038.5273; RP-HPLC: 100 % (t_R = 17.1 min, *k´* = 5.3); $C_{55}H_{67}N_{13}O_8$ (1037.52).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-[(3-propanoylaminopropyl)aminocarbonyl]argininamide ((*S*)-3.68). ^{1}H NMR (600 MHz, CD$_3$OD): δ = 1.11 (t, ^3J = 7.6 Hz, 3H, COCH$_2$C***H***$_3$), 1.51 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.52 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 1.67 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$NH), 2.18 (quat, ^3J = 7.6 Hz, 2H, COC***H***$_2$CH$_3$), 2.26 and 2.44 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.26 and 2.35 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.19 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$NH), 3.38-3.59 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.47 (br, 1H, 11-H

dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.46 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.86 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 10.5 ($COCH_2\boldsymbol{C}H_3$), 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 30.1 (***C***$^{\beta}$), 30.2 ($CO\boldsymbol{C}H_2CH_3$), 30.4 ($NHCH_2\boldsymbol{C}H_2CH_2NH$), 38.2 and 39.4 ($NH\boldsymbol{C}H_2CH_2\boldsymbol{C}H_2NH$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 41.8 (***C***$^{\delta}$), 44.6 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.9 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.2 (Ph), 126.2 (***C***$_{ar}$H), 128.2 (***C***$_{ar}$H), 129.5 (***C***$_{ar}$H), 129.9 (***C***$_{ar}$H), 130.2 (Ph), 130.4 (***C***$_{ar}$), 131.5 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***$ONPh)_2$), 155.4 (=N***C***ONH), 155.7 (N***C***(N)N), 171.4 (***C***=O lactam), 172.8 (***C***ON<), 174.5 (***C***$ONH(CH_2)_2N<$), 174.7 (***C***$ON^{\alpha}H$), 177.2 (***C***OCH_2CH_3) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1053 (100) $[M+H]^+$, 846 (30); HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{56}H_{70}N_{13}O_8$: 1053.5477, found: 1053.5430; RP-HPLC: 98 % (t_R = 18.3 min, *k´* = 5.8); $C_{56}H_{69}N_{13}O_8$ (1051.54).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-[(4-propanoylaminobutyl)aminocarbonyl]argininamide ((*S*)-**3.69).** Mp 127-130 °C; ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, $COCH_2C\boldsymbol{H}_3$), 1.51 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.51 (m, 4H, $NHCH_2(C\boldsymbol{H}_2)_2CH_2NH$), 1.52 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.74 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.59 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.17 (quat, ^3J = 7.6 Hz, 2H, $COC\boldsymbol{H}_2CH_3$), 2.26 and 2.44 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.26 and 2.35 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >$NCOC\boldsymbol{H}_2$-cyclopentyl), 3.15 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.16 (m, 4H, $NHC\boldsymbol{H}_2(CH_2)_2C\boldsymbol{H}_2NH$), 3.38-3.55 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.50 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.36 (m, 5H, Ph), 7.43 (m, 1H, ***H***$_{ar}$), 7.47 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.87 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 10.6 ($COCH_2\boldsymbol{C}H_3$), 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 27.7 and 27.8 ($NHCH_2(\boldsymbol{C}H_2)_2CH_2NH$), 30.0 (***C***$^{\beta}$), 30.2 ($CO\boldsymbol{C}H_2CH_3$), 38.4 ($NH\boldsymbol{C}H_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 39.9 and 40.4 ($NH\boldsymbol{C}H_2(CH_2)_2\boldsymbol{C}H_2NH$), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 41.8 (***C***$^{\delta}$), 44.6 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.8 (C-2/6 piperazine), 52.3 and 52.6 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.6 (***C***$_{ar}$H), 130.0 (***C***$_{ar}$H), 130.2 (Ph), 130.4 (***C***$_{ar}$), 131.5 (***C***$_{ar}$H), 132.1 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***$ONPh)_2$), 155.4 (=N***C***ONH), 155.8 (N***C***(N)N), 171.2 (***C***=O lactam), 172.8 (***C***ON<), 174.5 (***C***$ONH(CH_2)_2N<$), 174.7 (***C***$ON^{\alpha}H$), 177.1 (***C***OCH_2CH_3) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1067 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{57}H_{72}N_{13}O_8$: 1066.5627, found: 1066.5602; RP-HPLC: 99 % (t_R = 18.3 min, *k´* = 5.8); $C_{57}H_{71}N_{13}O_8$ (1065.55).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-[6-(propanoylaminohexyl)aminocarbonyl]argininamide ((*S*)-**3.70).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, $COCH_2CH_3$), 1.31 (m, 4H, $NH(CH_2)_2(CH_2)_2(CH_2)_2NH$), 1.48 (m, 4H, $NHCH_2CH_2(CH_2)_2CH_2CH_2NH$), 1.52 and 1.69 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.54 and 1.75 (m, 2H, $C^{\beta}H_2$), 1.60 (m, 2H, $C^{\gamma}H_2$), 2.16 (quat, ^3J = 7.6 Hz, 2H, $COCH_2CH_3$), 2.26 and 2.45 (m, 2H, $CH_2CON^{\alpha}H$), 2.35 and 2.44 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >NCOCH_2-cyclopentyl), 3.13 and 3.15 (m, 4H, $NHCH_2(CH_2)_4CH_2NH$), 3.15 (m, 2H, $C^{\delta}H_2$), 3.42-3.57 (m, 4H, 2/6-H piperazine), 3.45 and 3.56 (m, 2H, $NHCH_2CH_2N<$), 3.78 (m, 2H, $NHCH_2CH_2N<$), 4.25 (m, 1H, $C^{\alpha}H$), 4.63 (br, 1H, 11-H dibenzazepine-11-yl), 7.19 (m, 4H, H_{ar}), 7.32 (m, 5H, Ph), 7.36 (m, 5H, Ph), 7.45 (m, 1H, H_{ar}), 7.48 (m, 1H, H_{ar}), 7.56 (m, 1H, H_{ar}), 7.91 (m, 1H, H_{ar}) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 10.6 ($COCH_2CH_3$), 24.6 (C-3/4 cyclopentyl), 25.7 (C^{γ}), 27.4 and 27.5 ($NH(CH_2)_2(CH_2)_2(CH_2)_2NH$), 30.1 ($C^{\beta}$), 30.2 and 30.4 ($NHCH_2CH_2(CH_2)_2CH_2CH_2NH$), 30.3 ($COCH_2CH_3$), 38.4 ($NHCH_2CH_2N<$), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.8 (>NCOCH_2-cyclopentyl), 40.2 and 40.7 ($NHCH_2(CH_2)_4CH_2NH$), 41.2 ($NHCH_2CH_2N<$), 41.8 (C^{δ}), 44.6 ($CH_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 46.3 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.8 (C^{α}), 75.9 (C-11 dibenzazepine-11-yl), 123.1 ($C_{ar}H$), 124.2 (Ph), 126.3 ($C_{ar}H$), 128.2 ($C_{ar}H$), 129.9 ($C_{ar}H$), 130.2 (Ph), 130.8 ($C_{ar}H$), 131.7 ($C_{ar}H$), 132.3 ($C_{ar}H$), 133.6 ($C_{ar}H$), 137.1 (C_{ar}), 137.8 (C_{ar}), 154.5 ($N(CONPh)_2$), 155.4 (=$NCONH$), 155.8 ($NC(N)N$), 170.9 (C=O lactam), 172.8 ($CON<$), 174.6 ($CONH(CH_2)_2N<$), 174.7 ($CON^{\alpha}H$), 177.0 ($COCH_2CH_3$) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1095 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{59}H_{76}N_{13}O_8$: 1094.5940, found: 1094.5925; RP-HPLC: 99 % (t_R = 19.6 min, k' = 6.3); $C_{59}H_{75}N_{13}O_8$ (1093.59).

(2*S*)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-(6-propanoylaminohexanoyl)argininamide ((*S*)-**3.71).** MS (ES, +p) *m/z* (%): 1065 (100) $[M+H]^+$; HRMS(LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{58}H_{73}N_{12}O_8$: 1065.5674, found: 1065.5694; RP-HPLC: 88 % (t_R = 18.9 min, k' = 6.0); $C_{58}H_{72}N_{12}O_8$ (1064.56).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-(8-propanoylamino-3,6-dioxaoctanoyl)argininamide ((*S*)-**3.72).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.10 (t, ^3J = 7.6 Hz, 3H, $COCH_2CH_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.76 (m, 2H, $C^{\beta}H_2$), 1.63 (m, 2H, $C^{\gamma}H_2$), 2.18 (quat, ^3J = 7.6 Hz, 2H, $COCH_2CH_3$), 2.25 and 2.34 (m, 4H, 3/5-H piperazine), 2.26 and 2.44 (m, 2H, $CH_2CON^{\alpha}H$), 2.48 (m, 2H, >NCOCH_2-cyclopentyl), 3.21 (m, 2H, $C^{\delta}H_2$), 3.35 (t, ^3J = 5.6 Hz, 2H, OCH_2CH_2NH), 3.38-3.53 (m, 4H, 2/6-H piperazine), 3.46 and 3.55 (m, 2H, $NHCH_2CH_2N<$), 3.54 (t, ^3J = 5.6 Hz, 2H,

OC***H***$_2$CH$_2$NH), 3.66 and 3.73 (m, 4H, O(C***H***$_2$)$_2$O), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.20 (s, 2H, =NCOC***H***$_2$O), 4.26 (m, 1H, C$^{\alpha}$***H***), 4.48 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.46 (m, 1H, ***H***$_{ar}$), 7.54 (m, 1H, ***H***$_{ar}$), 7.87 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC): δ = 10.5 (COCH$_2$***C***H$_3$), 24.6 (C-3/4 cyclopentyl), 25.5 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.2 (CO***C***H$_2$CH$_3$), 38.4 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.5 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.2 and 42.0 (OCH$_2$***C***H$_2$NH), 41.2 (NHCH$_2$***C***H$_2$N<), 42.1 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.8 (C-1 cyclopentyl), 46.8 (C-2/6 piperazine), 52.3 and 52.6 (C-3/5 piperazine), 53.7 and 53.8 (***C***$^{\alpha}$), 70.7 (O***C***H$_2$CH$_2$NH), 71.2 (=NCO***C***H$_2$O), 71.2 and 72.2 (O(***C***H$_2$)$_2$O), 75.8 (C-11 dibenzazepine-11-yl), 123.1 (***C***$_{ar}$H), 124.2 (Ph), 126.2 (***C***$_{ar}$H), 128.2 (***C***$_{ar}$H), 129.5 (***C***$_{ar}$H), 129.9 (***C***$_{ar}$H), 130.2 (Ph), 130.4 (***C***$_{ar}$), 131.5 (***C***$_{ar}$H), 132.1 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.7 (N***C***(N)N), 171.3 (***C***=O lactam), 172.8 (***C***ON<), 173.9 (=N***C***OCH$_2$O), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H), 177.4 (***C***OCH$_2$CH$_3$) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1098 (100) [*M*+H]$^+$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{58}H_{73}N_{12}O_{10}$: 1097.5573, found: 1097.5531; RP-HPLC: 94 % (t_R = 18.4 min, *k´* = 5.8); $C_{58}H_{72}N_{12}O_{10}$ (1096.55).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-(15-propanoylamino-4,7,10,13-tetraoxapentadecanoyl)argininamide ((*S*)-**3.73).** ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC): δ = 1.10 (t, ^{3}*J* = 7.6 Hz, 3H, COCH$_2$C***H***$_3$), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.52 and 1.75 (m, 2H, C$^{\beta}$***H***$_2$), 1.60 (m, 2H, C$^{\gamma}$***H***$_2$), 2.18 (quat, ^{3}*J* = 7.6 Hz, 2H, COC***H***$_2$CH$_3$), 2.21 and 2.29 (m, 4H, 3/5-H piperazine), 2.25 and 2.43 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.48 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.69 (t, ^{3}*J* = 5.7 Hz, 2H, =NCOC***H***$_2$CH$_2$O), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.33 (t, ^{3}*J* = 5.6 Hz, 2H, OCH$_2$C***H***$_2$NH), 3.37-3.51 (m, 4H, 2/6-H piperazine), 3.44 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.51 (t, ^{3}*J* = 5.6 Hz, 2H, OC***H***$_2$CH$_2$NH), 3.60 (m, 12H, (O(C***H***$_2$)$_2$O)$_3$), 3.77 (m, 2H, =NCOCH$_2$C***H***$_2$O), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.41 (br, 1H, 11-H dibenzazepine-11-yl), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.85 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC): δ = 10.5 (COCH$_2$***C***H$_3$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.1 (CO***C***H$_2$CH$_3$), 38.4 and 38.5 (NH***C***H$_2$CH$_2$N<), 38.6 (=NCO***C***H$_2$CH$_2$O), 39.4 and 39.5 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.3 and 42.2 (OCH$_2$***C***H$_2$NH), 41.2 (NHCH$_2$***C***H$_2$N<), 42.0 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.8 and 45.9 (C-1 cyclopentyl), 47.0 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 53.7 and 53.8 (***C***$^{\alpha}$), 66.9 (=NCOCH$_2$***C***H$_2$O), 70.6 (O***C***H$_2$CH$_2$NH), 71.3 and 71.4 and 71.5 ((O(***C***H$_2$)$_2$O)$_3$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.2 (***C***$_{ar}$H), 129.4 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 130.3 (***C***$_{ar}$), 131.4 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.9 (N***C***(N)N), 171.5 (***C***=O lactam), 172.8 (***C***ON<), 174.5

(***C***ONH$(CH_2)_2$N<), 174.7 (***C***ON$^{\alpha}$H), 175.3 (=N***C***O$(CH_2)_2$O), 177.1 (***C***OCH_2CH_3) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1200 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{63}H_{83}N_{12}O_{12}$: 1199.6253, found: 1199.6267; RP-HPLC: 100 % (t_R = 18.2 min, *k´* = 5.7); $C_{63}H_{82}N_{12}O_{12}$ (1198.62).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{4-[2-(4-fluorobenzoylamino)ethyl]aminocarbonylbutanoyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-argininamide ((*S*)-**3.74).** ^{1}H NMR (300 MHz, CD_3OD): δ = 1.51 and 1.72 (m, 2H, C$^{\beta}$***H***$_2$), 1.51 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.62 (m, 2H, C$^{\gamma}$***H***$_2$), 1.90 (p, ^3J = 7.2 Hz, 2H, $COCH_2C\boldsymbol{H}_2CH_2CONH(CH_2)_2NH$), 2.20-2.37 (m, 6H, 3/5-H piperazine, $COC\boldsymbol{H}_2(CH_2)_2CONH(CH_2)_2NH$), 2.40-2.53 (m, 4H, >NCOC***H***$_2$-cyclopentyl, $CO(CH_2)_2C\boldsymbol{H}_2CONH(CH_2)_2NH$), 3.18 (m, 2H, C$^{\delta}$***H***$_2$), 3.35-3.62 (m, 10H, $CONHC\boldsymbol{H}_2C\boldsymbol{H}_2NH$, 2/6-H piperazine, $NHC\boldsymbol{H}_2CH_2N$<), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$<), 4.25 (m, 1H, C$^{\alpha}$***H***$_2$), 4.46 (br, 1H, 11-H dibenzazepine-11-yl), 7.13-7.23 (m, 6H, ***H***$_{ar}$, Ph), 7.32 (m, 5H, Ph), 7.36 (m, 7H, Ph, ***H***$_{ar}$), 7.51 (m, 1H, ***H***$_{ar}$), 7.85 (m, 3H, Ph, ***H***$_{ar}$) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 21.4 ($NCOCH_2\boldsymbol{C}H_2CH_2CONH$), 24.6 and 24.7 (C-3/4 cyclopentyl), 25.5 (***C***$^{\gamma}$), 30.1 (***C***$^{\beta}$), 35.7 (=NCO***C***$H_2(CH_2)_2CONH$), 36.9 ($NCO(CH_2)_2\boldsymbol{C}H_2CONH(CH_2)_2NH$), 37.8 ($CONHCH_2\boldsymbol{C}H_2NH$), 38.5 ($NH\boldsymbol{C}H_2CH_2N$<), 39.2 and 39.3 (C-2/5 cyclopentyl), 39.5 and 39.7 (>NCO***C***H_2-cyclopentyl), 40.1 ($CONH\boldsymbol{C}H_2CH_2NH$), 41.0 ($NHCH_2\boldsymbol{C}H_2N$<), 42.0 (***C***$^{\delta}$), 44.7 (***C***$H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 52.3 and 52.7 (C-3/5 piperazine), 53.8 (***C***$^{\alpha}$), 75.9 (C-11 dibenzazepine-11-yl), 116.3 (***C***$_{ar}$H), 116.6 (***C***$_{ar}$H), 123.1 (***C***$_{ar}$H), 124.2 (Ph), 126.2 (***C***$_{ar}$H), 128.2 (Ph), 129.4 (***C***$_{ar}$H), 129.9 (***C***$_{ar}$H), 130.3 (Ph), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 169.4 (***C***OC_6H_4F), 172.8 (***C***ON$^{\alpha}$H), 174.6 (***C***ONHC_2H_4N<), 174.8 (***C***ON<), 175.6 (=N***C***O$(CH_2)_3CONH$), 176.5 (=NCO$(CH_2)_3\boldsymbol{C}ONH$) ppm; MS (ES, +p) *m/z* (%): 1174 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{63}H_{73}FN_{13}O_9$: 1174.5638, found: 1174.5636; RP-HPLC: 95 % (t_R = 19.3 min, *k´* = 6.1); $C_{63}H_{72}FN_{13}O_9$ (1173.56).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{4-[3-(4-fluorobenzoylamino)propyl]aminocarbonylbutanoyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-argininamide ((*S*)-**3.75).** ^{1}H NMR (400 MHz, CD_3OD): δ = 1.50 and 1.70 (m, 2H, C$^{\beta}$***H***$_2$), 1.50 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.62 (m, 2H, C$^{\gamma}$***H***$_2$), 1.78 (p, ^3J = 6.8 Hz, 2H, $CONHCH_2C\boldsymbol{H}_2CH_2NH$), 1.93 (p, ^3J = 7.3 Hz, 2H, $COCH_2C\boldsymbol{H}_2CH_2CONH(CH_2)_3NH$), 2.24 and 2.29 (m, 4H, 3/5-H piperazine), 2.27 (m, 2H, $COC\boldsymbol{H}_2(CH_2)_2CONH(CH_2)_3NH$), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.50 (m, 2H, $CO(CH_2)_2C\boldsymbol{H}_2CONH(CH_2)_3NH$), 3.18 (m, 2H, C$^{\delta}$***H***$_2$), 3.25 (t, ^3J = 6.7 Hz, 2H, $CONHCH_2CH_2C\boldsymbol{H}_2NH$), 3.34 and 3.50 (m, 4H, 2/6-H piperazine), 3.39 (t, ^3J = 6.9 Hz, 2H, $CONHC\boldsymbol{H}_2CH_2CH_2NH$), 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N$<), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$<), 4.25

(m, 1H, $C^{\alpha}\boldsymbol{H}_2$), 4.40 (br, 1H, 11-H dibenzazepine-11-yl), 7.13 (m, 1H, $\boldsymbol{H}_{ar}$), 7.14 (m, 1H, $\boldsymbol{H}_{ar}$), 7.16 (m, 1H, $\boldsymbol{H}_{ar}$), 7.19 (m, 2H, Ph), 7.31 (m, 5H, Ph), 7.35 (m, 1H, $\boldsymbol{H}_{ar}$), 7.36 (m, 5H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.44 (m, 1H, $\boldsymbol{H}_{ar}$), 7.52 (m, 1H, $\boldsymbol{H}_{ar}$), 7.84 (m, 1H, $\boldsymbol{H}_{ar}$), 7.86 (m, 2H, Ph) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 21.4 ($NCOCH_2\boldsymbol{C}H_2CH_2CONH$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 30.3 ($CONHCH_2\boldsymbol{C}H_2CH_2NH$), 35.6 ($=NCO\boldsymbol{C}H_2(CH_2)_2CONH$), 36.9 ($=NCO(CH_2)_2\boldsymbol{C}H_2CONH$), 37.8 ($CONHCH_2CH_2\boldsymbol{C}H_2NH$), 38.4 ($CONH\boldsymbol{C}H_2CH_2CH_2NH$), 38.5 ($NH\boldsymbol{C}H_2CH_2N<$), 39.2 and 39.3 (C-2/5 cyclopentyl), 39.5 and 39.7 ($>NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 42.0 ($\boldsymbol{C}^{\delta}$), 42.2 und 47.0 (C-2/6 piperazine), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 52.2 and 52.7 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 ($\boldsymbol{C}_{ar}H$), 116.5 ($\boldsymbol{C}_{ar}H$), 123.0 ($\boldsymbol{C}_{ar}H$), 124.2 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.2 (Ph), 129.3 ($\boldsymbol{C}_{ar}H$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 131.3 ($\boldsymbol{C}_{ar}H$), 131.9 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.3 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 142.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.0 (NC(N)N), 167.4 ($\boldsymbol{C}_{ar}F$), 169.1 ($\boldsymbol{C}OC_6H_4F$), 171.6 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON^{\alpha}H$), 174.5 ($\boldsymbol{C}ONHC_2H_4N<$), 174.7 ($\boldsymbol{C}ON<$), 175.2 ($=N\boldsymbol{C}O(CH_2)_3CONH$), 176.5 ($=NCO(CH_2)_3\boldsymbol{C}ONH$) ppm; MS (ES, +p) *m/z* (%): 1188 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{64}H_{75}FN_{13}O_9$: 1188.5795, found: 1188.5807; RP-HPLC: 100 % (t_R = 19.6 min, k' = 6.3); $C_{64}H_{74}FN_{13}O_9$ (1187.57).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-[18-(4-fluoro-benzoylamino)-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*S*)-**3.76).** ^{1}H NMR (600 MHz, CD_3OD, HSQC): δ = 1.50 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.50 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.50 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.58 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 1.71 (m, 2H, $NHCH_2C\boldsymbol{H}_2CH_2O$), 1.86 (m, 2H, $OCH_2C\boldsymbol{H}_2CH_2NHCOC_6H_4F$), 2.26 and 2.42 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.19 and 2.26 (m, 4H, 3/5-H piperazine), 2.47 (m, 2H, $>NCOC\boldsymbol{H}_2$-cyclopentyl), 2.55 (m, 2H, $=NCOC\boldsymbol{H}_2CH_2CONH$), 2.68 (m, 2H, $=NCOCH_2C\boldsymbol{H}_2CONH$), 3.16 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.22 (m, 2H, $NHC\boldsymbol{H}_2(CH_2)_2O$), 3.35-3.49 (m, 4H, 2/6-H piperazine), 3.43 and 3.57 (m, 2H, $NHC\boldsymbol{H}_2CH_2N<$), 3.46 (m, 2H, $O(CH_2)_2C\boldsymbol{H}_2NHCOC_6H_4F$), 3.46 and 3.57 (m, 4H, $(NH(CH_2)_2C\boldsymbol{H}_2O)_2$) 3.53 and 3.59 (m, 8H, $(O(C\boldsymbol{H}_2)_2O)_2$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N<$), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.36 (br, 1H, 11-H dibenzazepine-11-yl), 7.16 (m, 6H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.36 (m, 5H, Ph), 7.40 (m, 1H, $\boldsymbol{H}_{ar}$), 7.44 (m, 1H, $\boldsymbol{H}_{ar}$), 7.51 (m, 1H, $\boldsymbol{H}_{ar}$), 7.84 (m, 3H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 24.6 and 24.7 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 30.4 ($(NHCH_2\boldsymbol{C}H_2CH_2O)_2$), 30.4 ($=NCO\boldsymbol{C}H_2CH_2CO$), 33.0 ($=NCOCH_2\boldsymbol{C}H_2CO$), 38.0 ($NH\boldsymbol{C}H_2(CH_2)_2O$), 38.5 ($NH\boldsymbol{C}H_2CH_2N<$), 38.8 ($O(CH_2)_2\boldsymbol{C}H_2NHCOC_6H_4F$), 39.3 and 39.5 (C-2/5 cyclopentyl), 39.7 ($>NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N<$), 42.1 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 52.3 and 52.7 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 69.9 and 70.2 ($(NH(CH_2)_2\boldsymbol{C}H_2O)_2$), 71.3 and 71.5 ($(O(\boldsymbol{C}H_2)_2O)_2$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 ($\boldsymbol{C}_{ar}H$), 116.5 ($\boldsymbol{C}_{ar}H$), 123.0 ($\boldsymbol{C}_{ar}H$), 124.2 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.2 ($\boldsymbol{C}_{ar}H$), 129.2 ($\boldsymbol{C}_{ar}H$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 130.9 ($\boldsymbol{C}_{ar}H$), 131.3 ($\boldsymbol{C}_{ar}H$), 131.9 ($\boldsymbol{C}_{ar}H$), 132.2 ($\boldsymbol{C}_{ar}$),

132.4 (***C***$_{ar}$), 133.2 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.9 (N***C***(N)N), 165.3 (***C***$_{ar}$F), 167.0 (***C***OC$_6$H$_4$F), 171.7 (***C***=O lactam), 172.8 (***C***ON$^{\alpha}$H), 173.7 (=NCO(CH$_2$)$_2$***C***O), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON<), 176.2 (=N***C***O(CH$_2$)$_2$CO) ppm; MS (ES, +p) *m/z* (%): 1321 (5) [*M*+H]$^+$, 661 (100) [*M*+2H]$^{2+}$; HRMS (ESI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{70}H_{87}FN_{13}O_{12}$: 1320.6576, found: 1320.6572; RP-HPLC: 96 % (t_R = 20.1 min, *k´* = 6.4); $C_{70}H_{86}FN_{13}O_{12}$ (1319.65).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{[3-(4-fluorobenzoylamino)propyl]aminocarbonyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.77).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.50 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.52 and 1.66 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.73 (m, 2H, C$^{\beta}$***H***$_2$), 1.61 (m, 2H, C$^{\gamma}$***H***$_2$), 1.80 (p, ^3J = 6.6 Hz, 2H, NHCH$_2$C***H***$_2$CH$_2$NH), 2.25 and 2.35 (m, 4H, 3/5-H piperazine), 2.26 and 2.44 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.47 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.14 (m, 2H, C$^{\delta}$***H***$_2$), 3.26 and 3.40 (t, ^3J = 6.4 Hz, 2H, t, ^3J = 6.8 Hz, 2H, NHC***H***$_2$CH$_2$C***H***$_2$NH), 3.38-3.58 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***),4.44 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, 4/5-H 4-fluorobenzoyl), 7.17 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.36 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.45 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.86 (m, 3H, ***H***$_{ar}$, 2/6-H 4-fluorobenzoyl) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.4 (NHCH$_2$***C***H$_2$CH$_2$NH), 38.2 and 38.3 (NH***C***H$_2$CH$_2$***C***H$_2$NH), 38.5 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 41.2 (NHCH$_2$***C***H$_2$N<), 41.9 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 46.9 (C-2/6 piperazine), 52.3 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 and 116.5 (C-3/5 4-fluorobenzoyl), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.2 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.4 (C-2/6 4-fluorobenzoyl), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 130.8 (***C***$_{ar}$), 131.4 (C-1 4-fluorobenzoyl), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.4 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.5 (=N***C***ONH), 155.7 (N***C***(N)N), 167.0 (C-4 4-fluorobenzoyl), 169.2 (***C***=O 4-fluorobenzoyl), 171.5 (***C***=O lactam), 172.8 (***C***ON<), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1119 (100) [*M*+H]$^+$, 912 (50); HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{60}H_{69}FN_{13}O_8$: 1118.5376, found: 1118.5348; RP-HPLC: 98 % (t_R = 20.7 min, *k´* = 6.7); $C_{60}H_{68}FN_{13}O_8$ (1117.53).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{[4-(4-fluorobenzoylamino)butyl]aminocarbonyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.78).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.50 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.50 (m, 4H, NHCH$_2$(C***H***$_2$)$_2$CH$_2$NH), 1.52 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.73 (m, 2H, C$^{\beta}$***H***$_2$), 1.59 (m, 2H, C$^{\gamma}$***H***$_2$), 2.10 and 2.19 (m, 4H, 3/5-H piperazine), 2.19 and 2.40 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.47 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.12 (m, 2H, C$^{\delta}$***H***$_2$), 3.22

and 3.37 (t, ^{3}J = 6.3 Hz, 2H, t, ^{3}J = 6.7 Hz, 2H, NHC***H***$_2$(CH$_2$)$_2$C***H***$_2$NH), 3.34-3.58 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 2H, C$^{\alpha}$***H***, 11-H dibenzazepine-11-yl), 7.13 (m, 2H, 4/5-H 4-fluorobenzoyl), 7.16 (m, 2H, ***H***$_{ar}$), 7.19 (m, 2H, ***H***$_{ar}$), 7.32 (m, 6H, Ph), 7.36 (m, 5H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.49 (m, 1H, ***H***$_{ar}$), 7.80 (d, ^{3}J = 7.5 Hz, 1H, ***H***$_{ar}$), 7.85 (m, 2H, 2/6-H 4-fluorobenzoyl) ppm; ^{13}C NMR (150 MHz, CD$_3$OD): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 27.8 and 27.9 (NHCH$_2$(***C***H$_2$)$_2$CH$_2$NH), 30.0 (***C***$^{\beta}$), 38.5 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.5 and 42.6 (NH***C***H$_2$(CH$_2$)$_2$***C***H$_2$NH), 41.2 (NHCH$_2$***C***H$_2$N<), 41.9 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 47.5 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 and 116.4 (C-3/5 4-fluorobenzoyl), 122.8 (Ph), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.0 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.0 and 129.4 (C-2/6 4-fluorobenzoyl), 129.9 (***C***$_{ar}$H), 130.0 (***C***$_{ar}$H), 130.2 (Ph), 130.8 (***C***$_{ar}$), 131.1 (C-1 4-fluorobenzoyl), 131.7 (***C***$_{ar}$H), 132.1 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.1 (***C***$_{ar}$H), 136.8 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.4 (=N***C***ONH), 155.7 (N***C***(N)N), 167.0 (C-4 4-fluorobenzoyl), 169.0 (***C***=O 4-fluorobenzoyl), 172.0 (***C***=O lactam), 172.7 (***C***ON<), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1133 (100) [*M*+H]$^+$, 928 (50); HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for C$_{61}$H$_{71}$FN$_{13}$O$_8$: 1132.5533, found: 1132.5541; RP-HPLC: 98 % (t_R = 20.8 min, *k´* = 6.7); C$_{61}$H$_{70}$FN$_{13}$O$_8$ (1131.55).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{[6-(4-fluorobenzoylamino)hexyl]aminocarbonyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.79).** ^{1}H NMR (600 MHz, CD$_3$OD): δ = 1.36 (m, 4H, NH(CH$_2$)$_2$(C***H***$_2$)$_2$(CH$_2$)$_2$NH), 1.49 (m, 4H, NHCH$_2$C***H***$_2$(CH$_2$)$_2$C***H***$_2$CH$_2$NH), 1.50 and 1.62 (m, 4H, 3/4-H cyclopentyl), 1.50 and 1.68 (m, 4H, 2/5-H cyclopentyl), 1.52 and 1.73 (m, 2H, C$^{\beta}$***H***$_2$), 1.61 (m, 2H, C$^{\gamma}$***H***$_2$), 2.23 and 2.46 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.28 and 2.41 (m, 4H, 3/5-H piperazine), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.15 and 3.35 (m, 4H, NHC***H***$_2$(CH$_2$)$_4$C***H***$_2$NH), 3.16 (m, 2H, C$^{\delta}$***H***$_2$), 3.36-3.59 (m, 4H, 2/6-H piperazine), 3.45 and 3.55 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.77 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.32 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, 4/5-H 4-fluorobenzoyl), 7.15 (m, 1H, ***H***$_{ar}$), 7.17 (m, 1H, ***H***$_{ar}$), 7.18 (m, 2H, ***H***$_{ar}$), 7.28-7.40 (m, 11H), 7.42 (m, 1H, ***H***$_{ar}$), 7.50 (m, 1H, ***H***$_{ar}$), 7.84 (m, 3H, 2/6-H 4-fluorobenzoyl, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.7 (***C***$^{\gamma}$), 27.4 and 27.6 (NH(CH$_2$)$_2$(***C***H$_2$)$_2$(CH$_2$)$_2$NH), 30.0 (***C***$^{\beta}$), 30.4 (NHCH$_2$***C***H$_2$(CH$_2$)$_2$***C***H$_2$CH$_2$NH), 38.5 (NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>NCO***C***H$_2$-cyclopentyl), 40.7 and 42.4 (NH***C***H$_2$(CH$_2$)$_4$***C***H$_2$NH), 41.2 (NHCH$_2$***C***H$_2$N<), 41.8 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 and 116.4 (C-3/5 4-fluorobenzoyl), 122.6 (Ph), 123.0 (***C***$_{ar}$H), 124.1 (Ph), 126.1 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.2 and 129.6 (C-2/6 4-fluorobenzoyl), 130.2 (Ph), 130.8 (***C***$_{ar}$), 131.2 (C-1 4-fluorobenzoyl), 131.8 (***C***$_{ar}$H), 132.2 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.2 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8

(***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 155.4 (=N***C***ONH), 155.7 (N***C***(N)N), 167.0 (C-4 4-fluorobenzoyl), 169.0 (***C***=O 4-fluorobenzoyl), 171.8 (***C***=O lactam), 172.7 (***C***ON<), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON$^{\alpha}$H) ppm; MS (LSI, MeOH/gly) *m/z* (%): 1161 (100) [*M*+H]$^+$, 954 (40); HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{63}H_{75}FN_{13}O_8$: 1160.5846, found: 1160.5816; RP-HPLC: 98 % (t_R = 22.1 min, *k´* = 7.2); $C_{63}H_{74}FN_{13}O_8$ (1159.58).

(2*S*)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-[6-(4-fluoro-benzoylamino)hexanoyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo-[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-3.80). $[\alpha]_D^{20}$= –4.7 cm^3 g^{-1} dm^{-1} (c = 0.71 in CH$_3$CN/H$_2$O 10:1); ^{1}H NMR (600 MHz, DMSO-d$_6$, HSQC): δ= 1.32 (m, 2H, C***H***$_2$(CH$_2$)$_2$NH), 1.48 (m, 2H, C$^{\gamma}$***H***$_2$), 1.44 and 1.63 (m, 2H, C$^{\beta}$***H***$_2$), 1.41-1.63 (m, 4H, 2/5-H cyclopentyl), 1.52 (m, 2H, C***H***$_2$CH$_2$NHBz(4F)), 1.55 (m, 4H, 3/4-H cyclopentyl), 1.57 (m, 2H, COCH$_2$C***H***$_2$), 2.16 and 2.18 (m, 4H, 3/5-H piperazine), 2.21 and 2.32 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.41 (t, ^3J = 7.3 Hz, 2H, C(O)C***H***$_2$CH$_2$), 2.42 and 2.50 (m, 2H, NCOC***H***$_2$-cyclopentyl), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.25 (m, 2H, C***H***$_2$NHBz(4F)), 3.33-3.37 (m, 4H, 2/6-H piperazine), 3.31 and 3.40 (m, 2H, NHC***H***$_2$CH$_2$N), 3.60 (t, ^3J = 6.0 Hz, 2H, NHCH$_2$C***H***$_2$N), 4.17 (m, 1H, C$^{\alpha}$***H***), 4.43 (br, 1H, 11-H dibenzazepine-11-yl), 7.08 (m, 1H, ***H***$_{ar}$), 7.14 (m, 1H, ***H***$_{ar}$), 7.22 (m, 2H, Ph), 7.28 (m, 1H, ***H***$_{ar}$), 7.35 (m, 1H, ***H***$_{ar}$), 7.37 (m, 8H, Ph), 7.41 (m, 1H, ***H***$_{ar}$), 7.43 (m, 1H, ***H***$_{ar}$), 7.52 (m, 1H, ***H***$_{ar}$), 7.78 (m, 1H, ***H***$_{ar}$), 7.98 (d, ^3J = 8.0 Hz, 1H, N$^{\alpha}$***H***), 8.24 (t, ^3J = 5.7 Hz, 1H, CON***H***C$_2$H$_4$N), 8.48 (t, ^3J = 5.5 Hz, 1H, N***H***Bz(4F)), 8.68 and 8.81 (br, 2H, N$^{\omega}$***H***$_2$), 9.20 (t, ^3J = 5.6 Hz, 1H, N$^{\delta}$***H***), 10.40 (s, 1H, C(O)N***H*** lactam), 11.83 (s, 1H, N$^{\omega'}$***H***) ppm; ^{13}C NMR (150 MHz, DMSO-d$_6$, HSQC): δ = 23.3 (C-3/4 cyclopentyl), 23.6 (COCH$_2$***C***H$_3$), 24.1 (***C***$^{\gamma}$), 25.7 (***C***H$_2$(CH$_2$)$_2$NH), 28.7 (***C***$^{\beta}$), 28.8 (CH$_2$***C***H$_2$CH$_2$NH), 36.0 (C(O)***C***H$_2$), 36.1 (NH***C***H$_2$CH$_2$N), 37.2 (C-2/5 cyclopentyl), 38.5 (NCO***C***H$_2$-cyclopentyl), 39.0 (***C***H$_2$NHBz(4F)), 39.6 (NHCH$_2$***C***H$_2$N), 40.5 (***C***$^{\delta}$), 40.4 and 44.9 (C-2/6 piperazine), 42.8 (***C***H$_2$CON$^{\alpha}$H), 44.0 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.7 (***C***$^{\alpha}$), 73.6 (C-11 dibenzazepine-11-yl), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 121.3 (***C***$_{ar}$H), 122.6 (Ph), 123.8 (***C***$_{ar}$H), 126.5 (Ph), 128.1 (***C***$_{ar}$H), 128.4 (***C***$_{ar}$H), 128.7 (***C***$_{ar}$H), 128.9 (Ph), 129.7 (d, $^3J_{C,F}$ = 8.9 Hz), 130.2 (***C***$_{ar}$H), 130.7 (***C***$_{ar}$H), 131.1 (d, $^4J_{C,F}$ = 2.9 Hz), 131.5 (***C***$_{ar}$H), 131.1 (***C***$_{ar}$), 136.2 (***C***$_{ar}$), 136.5 (***C***$_{ar}$), 141.5 (***C***$_{ar}$), 152.5 (N(***C***ONPh)$_2$), 152.9 (NC(N)N), 163.7 (d, $^1J_{C,F}$ = 248.0 Hz), 165.0 (***C***OC$_6$H$_4$F), 167.9 (***C***=O lactam), 170.0 (***C***ON), 171.2 (***C***ON$^{\alpha}$H), 171.9 (***C***ONHC$_2$H$_4$N), 175.2 (***C***O(CH$_2$)$_5$) ppm; IR (neat): υ´ = 3300, 2943, 1641, 1443, 1289, 1172 cm^{-1}; MS (ES, +p) *m/z* (%): 1132 (100) [*M*+H]$^+$; RP-HPLC: 98 % (t_R = 22.1 min, *k´* = 7.8); anal. calcd. for $C_{62}H_{71}FN_{12}O_8$·2 H$_2$O·2 HCl: C 60.04, H 6.26, N 13.56 %, found: C 60.27, H 6.55, N 13.51 %; $C_{62}H_{71}FN_{12}O_8$ (1131.30).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-[8-(4-fluoro-benzoylamino)-3,6-dioxaoctanoyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide ((*S*)-**3.81).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.50 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.75 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.63 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.12 and 2.39 (m, 4H, 3/5-H piperazine), 2.26 and 2.44 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 2H, >NCOC$\boldsymbol{H}_2$-cyclopentyl), 3.18 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.34-3.49 (m, 4H, 2/6-H piperazine), 3.43 and 3.53 (m, 2H, NHC$\boldsymbol{H}_2CH_2N$<), 3.57 (t, ^{3}J = 5.6 Hz, 2H, $OCH_2C\boldsymbol{H}_2NH$), 3.66 (t, ^{3}J = 5.6 Hz, 2H, O$\boldsymbol{C}H_2CH_2NH$), 3.70 and 3.75 (m, 4H, $O(C\boldsymbol{H}_2)_2O$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$<), 4.19 (s, 2H, $COC\boldsymbol{H}_2O$), 4.26 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.28 (br, 1H, 11-H dibenzazepine-11-yl), 7.13 (m, 2H, 4/5-H 4-fluorobenzoyl), 7.15 (m, 1H, $\boldsymbol{H}_{ar}$), 7.16 (m, 1H, $\boldsymbol{H}_{ar}$), 7.19 (m, 2H, $\boldsymbol{H}_{ar}$), 7.27-7.40 (m, 11H), 7.42 (m, 1H, $\boldsymbol{H}_{ar}$), 7.50 (m, 1H, $\boldsymbol{H}_{ar}$), 7.81 (d, ^{3}J = 7.6 Hz, 1H, $\boldsymbol{H}_{ar}$), 7.85 (m, 2H, 2/6-H 4-fluorobenzoyl) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 38.5 ($NH\boldsymbol{C}H_2CH_2N$<), 39.3 and 39.5 (C-2/5 cyclopentyl), 39.6 (>NCO$\boldsymbol{C}H_2$-cyclopentyl), 40.8 and 42.5 ($OCH_2\boldsymbol{C}H_2NH$), 41.2 ($NHCH_2\boldsymbol{C}H_2N$<), 42.1 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 47.4 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 70.6 (O$\boldsymbol{C}H_2CH_2NH$), 71.2 (=NCO$\boldsymbol{C}H_2O$), 71.2 and 72.2 ($O(\boldsymbol{C}H_2)_2O$), 75.8 (C-11 dibenzazepine-11-yl), 116.4 and 116.5 (C-3/5 4-fluorobenzoyl), 122.9 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.0 (C-2/6 4-fluorobenzoyl), 130.2 (Ph), 130.9 ($\boldsymbol{C}_{ar}$), 131.2 (C-1 4-fluorobenzoyl), 131.7 ($\boldsymbol{C}_{ar}H$), 132.0 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.1 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 154.6 ($N\boldsymbol{C}(N)N$), 167.0 (C-4 4-fluorobenzoyl), 169.3 ($\boldsymbol{C}$=O 4-fluorobenzoyl), 171.9 ($\boldsymbol{C}$=O lactam), 172.7 ($\boldsymbol{C}$ON<), 173.8 (=N$\boldsymbol{C}OCH_2O$), 174.5 ($\boldsymbol{C}ONH(CH_2)_2N$<), 174.7 ($\boldsymbol{C}ON^{\alpha}H$) ppm; MS (ES, +p) *m/z* (%): 1164 (50) $[M+H]^+$, 582 (100) $[M+2H]^{2+}$; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd. for $C_{62}H_{72}FN_{12}O_{10}$: 1163.5473, found: 1163.5492; RP-HPLC: 97 % (t_R = 20.7 min, *k´* = 6.7); $C_{62}H_{71}FN_{12}O_{10}$ (1162.54).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-[15-(4-fluoro-benzoylamino)-4,7,10,13-tetraoxapentadecanoyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-argininamide ((*S*)-**3.82).** Mp 112-115 °C; ^{1}H NMR (600 MHz, CD_3OD): δ = 1.50 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.51 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.53 and 1.75 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.63 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.12 and 2.39 (m, 4H, 3/5-H piperazine), 2.26 and 2.44 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 2H, >NCOC$\boldsymbol{H}_2$-cyclopentyl), 2.66 (t, ^{3}J = 5.7 Hz, 2H, =NCOC$\boldsymbol{H}_2CH_2O$), 3.17 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.34-3.49 (m, 4H, 2/6-H piperazine), 3.43 and 3.53 (m, 2H, NHC$\boldsymbol{H}_2CH_2N$<), 3.56 (m, 2H, $OCH_2C\boldsymbol{H}_2NH$), 3.56 and 3.63 (m, 12H, $(O(C\boldsymbol{H}_2)_2O)_3$), 3.59 (m, 2H, OC$\boldsymbol{H}_2CH_2NH$), 3.74 (t, ^{3}J = 5.7 Hz, 2H, $=NCOCH_2C\boldsymbol{H}_2O$), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$<), 4.26 (m, 2H, $C^{\alpha}\boldsymbol{H}$, 11-H dibenzazepine-11-yl), 7.13 (m, 2H, 4/5-H 4-fluorobenzoyl), 7.15 (m, 1H, $\boldsymbol{H}_{ar}$), 7.16 (m, 1H, $\boldsymbol{H}_{ar}$), 7.19 (m, 2H, $\boldsymbol{H}_{ar}$), 7.27-7.40 (m, 11H), 7.42 (m, 1H, $\boldsymbol{H}_{ar}$), 7.50 (m, 1H, $\boldsymbol{H}_{ar}$), 7.81 (d, ^{3}J = 7.6 Hz, 1H, $\boldsymbol{H}_{ar}$),7.87 (m, 2H, 2/6-H 4-fluorobenzoyl) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 24.5 and 24.6 (C-3/4

cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 38.5 (NH***C***H_2CH_2N<), 38.6 (=NCO***C***H_2CH_2O), 39.3 and 39.5 (C-2/5 cyclopentyl), 39.6 (>NCO***C***H_2-cyclopentyl), 41.0 and 42.5 (OCH_2***C***H_2NH), 41.2 ($NHCH_2$***C***H_2N<), 42.1 (***C***$^{\delta}$), 44.7 (***C***$H_2CON^{\alpha}H$), 45.9 (C-1 cyclopentyl), 47.4 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 66.9 (=$NCOCH_2$***C***H_2O), 70.5 (O***C***H_2CH_2NH), 71.3 and 71.4 and 71.5 ($(O(\mathbf{C}H_2)_2O)_3$), 75.8 (C-11 dibenzazepine-11-yl), 116.3 and 116.5 (C-3/5 4-fluorobenzoyl), 122.9 (***C***$_{ar}$H), 124.1 (Ph), 126.0 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.0 (C-2/6 4-fluorobenzoyl), 129.5 (***C***$_{ar}$H), 130.0 (***C***$_{ar}$H), 130.2 (Ph), 130.9 (***C***$_{ar}$), 131.2 (C-1 4-fluorobenzoyl), 131.7 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.1 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***$ONPh)_2$), 154.8 (N***C***(N)N), 167.0 (C-4 4-fluorobenzoyl), 169.1 (***C***=O 4-fluorobenzoyl), 171.9 (***C***=O lactam), 172.7 (***C***ON<), 174.5 (***C***$ONH(CH_2)_2N$<), 174.7 (***C***$ON^{\alpha}H$), 175.2 (=N***C***OCH_2CH_2O) ppm; MS (ES, +p) *m/z* (%): 1266 (25) $[M+H]^+$, 633 (100) $[M+2H]^{2+}$; HRMS (ESI-MS): *m/z* $[M+H]^+$ calcd. for $C_{67}H_{82}FN_{12}O_{12}$: 1265.6154, found: 1265.6171; RP-HPLC: 99 % (t_R = 20.7 min, *k´* = 6.7); $C_{67}H_{81}FN_{12}O_{12}$ (1264.61).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-{4-[3-(2-fluoropropanoylamino)propyl]aminocarbonylbutanoyl}-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-argininamide ((*S*)-**3.83).** Mp 129 °C; ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.50 (d, ^3J = 6.8 Hz, 3H, COCHFC***H***$_3$), 1.51 and 1.74 (m, 2H, C$^{\beta}$***H***$_2$), 1.50 and 1.69 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.60 (m, 2H, C$^{\gamma}$***H***$_2$), 1.69 (p, ^3J = 6.8 Hz, 2H, $CONHCH_2C$***H***$_2CH_2NH$), 1.92 (p, ^3J = 7.3 Hz, 2H, =$NCOCH_2C$***H***$_2CH_2CONH$), 2.24 and 2.31 (m, 4H, 3/5-H piperazine), 2.25 (m, 2H, =NCOC***H***$_2(CH_2)_2CONH$), 2.26 and 2.43 (m, 2H, C***H***$_2CON^{\alpha}H$), 2.48 (m, 2H, =$NCO(CH_2)_2C$***H***$_2CONH$), 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 3.17 (m, 2H, C$^{\delta}$***H***$_2$), 3.20 (t, ^3J = 6.7 Hz, 2H, CONHC***H***$_2(CH_2)_2NH$), 3.25 (t, ^3J = 6.9 Hz, 2H, $CONH(CH_2)_2C$***H***$_2NH$), 3.45 and 3.54 (m, 4H, 2/6-H piperazine), 3.45 and 3.56 (m, 2H, NHC***H***$_2CH_2N$<), 3.78 (m, 2H, $NHCH_2C$***H***$_2N$<), 4.25 (m, 1H, C$^{\alpha}$***H***$_2$), 4.43 (br, 1H, 11-H dibenzazepine-11-yl), 4.96 (quat, ^3J = 6.8 Hz, 1H, COC***H***FCH_3), 7.15 (m, 2H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.46 (m, 1H, ***H***$_{ar}$), 7.53 (m, 1H, ***H***$_{ar}$), 7.85 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HCQC): $^{\delta}$ = 18.9 and 19.0 (COCHF***C***H_3), 21.4 (=$NCOCH_2$***C***H_2CH_2CONH), 24.5 und 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.2 ($CONHCH_2$***C***H_2CH_2NH), 35.6 (=NCO***C***$H_2(CH_2)_2CONH$), 36.9 (=$NCO(CH_2)_2$***C***H_2CONH), 37.4 ($CONH(CH_2)_2$***C***H_2NH), 37.6 (CONH***C***$H_2(CH_2)_2NH$), 38.4 (NH***C***H_2CH_2N<), 39.3 (C-2/5 cyclopentyl), 39.5 and 39.7 (>NCO***C***H_2cyclopentyl), 41.2 ($NHCH_2$***C***H_2N<), 42.0 (***C***$^{\delta}$), 44.6 (***C***$H_2CON^{\alpha}H$), 47.0 (C-2/6 piperazine), 45.8 (C-1 cyclopentyl), 52.2 und 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 88.8 and 90.0 (CO***C***$HFCH_3$), 123.0 (***C***$_{ar}$H), 124.2 (Ph), 126.2 (***C***$_{ar}$H), 128.2 (***C***$_{ar}$H), 129.4 (***C***$_{ar}$H), 129.8 (***C***$_{ar}$H), 130.2 (Ph), 130.3 (***C***$_{ar}$), 131.4 (***C***$_{ar}$H), 132.0 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.3 (***C***$_{ar}$H), 137.0 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***$ONPh)_2$), 155.0 (NC(N)N), 172.8 (***C***$ON^{\alpha}H$), 173.4 and 173.5 (***C***$OCHFCH_3$), 174.5 (***C***$ONHC_2H_4N$<), 174.7 (***C***ON<), 175.2 (=N***C***$O(CH_2)_3CONH$), 176.4 (=$NCO(CH_2)_3$***C***ONH) ppm; MS (ES, +p) *m/z*

(%): 1141 (30) [*M*+H]$^+$, 571 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{60}H_{75}FN_{13}O_9$: 1140.5795, found: 1140.5799; RP-HPLC: 99 % (t_R = 17.7 min, *k´* = 5.6); $C_{60}H_{74}FN_{13}O_9$ (1139.57).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-[18-(2-fluoro-propanoylamino)-4-oxo-9,12,15-trioxa-5-azaoctadecanoyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide ((*S*)-3.84). ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC): δ = 1.47 and 1.50, (2x d, ^3J = 6.8 Hz, 3H, CFHC***H***$_3$), 1.50 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.50 and 1.67 (m, 4H, 2/5-H cyclopentyl), 1.50 (m, 2H, C$^{\beta}$***H***$_2$), 1.58 (m, 2H, C$^{\gamma}$***H***$_2$), 1.73 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$O), 1.77 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$O), 2.14 and 2.21 (m, 4H, 3/5-H piperazine), 2.23 and 2.41 (m, 2H, C***H***$_2$CON$^{\alpha}$H), 2.47 and 2.49 (m, 2H, >NCOC***H***$_2$-cyclopentyl), 2.55 and 2.70 (2x m, 4H, =NCO(C***H***$_2$)$_2$CONH), 3.16 (m, 2H, C$^{\delta}$***H***$_2$), 3.24 (t, ^3J = 6.8 Hz, 2H, NHC***H***$_2$(CH$_2$)$_2$O), 3.31 (m, 2H, NHC***H***$_2$(CH$_2$)$_2$), 3.32-3.49 (m, 4H, 2/6-H piperazine), 3.44 and 3.53 (m, 2H, NHC***H***$_2$CH$_2$N<), 3.49 (m, 2H, NH(CH$_2$)$_2$C***H***$_2$O), 3.51 (m, 2H, NH(CH$_2$)$_2$C***H***$_2$O), 3.56 and 3.61 (m, 8H, (O(C***H***$_2$)$_2$O)$_2$), 3.78 (m, 2H, NHCH$_2$C***H***$_2$N<), 4.25 (m, 1H, C$^{\alpha}$***H***), 4.28 (br, 1H, 11-H dibenzazepine-11-yl), 4.90 and 4.98 (2x quat, ^3J = 6.8 Hz, 1H, C***H***FCH$_3$), 7.12 (m, 1H, ***H***$_{ar}$), 7.13 (m, 1H, ***H***$_{ar}$), 7.20 (m, 2H, ***H***$_{ar}$), 7.32 (m, 5H, Ph), 7.33 (m, 1H, ***H***$_{ar}$), 7.38 (m, 5H, Ph), 7.42 (m, 1H, ***H***$_{ar}$), 7.50 (m, 1H, ***H***$_{ar}$), 7.81 (m, 1H, ***H***$_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC): δ = 18.9 (COCHF***C***H$_3$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 (***C***$^{\gamma}$), 30.0 (***C***$^{\beta}$), 30.3 and 30.4 (2x NHCH$_2$***C***H$_2$CH$_2$O), 30.3 (=NCO***C***H$_2$CH$_2$CO), 33.0 (=NCOCH$_2$***C***H$_2$CO), 37.8 and 38.0 (2x NH***C***H$_2$(CH$_2$)$_2$O), 38.5 (NH***C***H$_2$CH$_2$N<), 39.3 (C-2/5 cyclopentyl), 39.6 (>NCO***C***H$_2$-cyclopentyl), 41.2 (NHCH$_2$***C***H$_2$N<), 42.0 (***C***$^{\delta}$), 44.7 (***C***H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 47.5 (C-2/6 piperazine), 52.2 and 52.6 (C-3/5 piperazine), 53.7 (***C***$^{\alpha}$), 69.9 and 70.1 (2x HN(CH$_2$)$_2$***C***H$_2$O), 71.2 and 71.3 ((O(***C***H$_2$)$_2$O)$_2$), 75.8 (C-11 dibenzazepine-11-yl), 88.8 and 90.0 (CO***C***HFCH$_3$), 122.9 (***C***$_{ar}$H), 124.1 (Ph), 126.0 (***C***$_{ar}$H), 128.1 (***C***$_{ar}$H), 129.0 (***C***$_{ar}$H), 129.4 (***C***$_{ar}$H), 130.0 (***C***$_{ar}$), 130.2 (Ph), 131.2 (***C***$_{ar}$H), 131.7 (***C***$_{ar}$H), 132.4 (***C***$_{ar}$), 133.1 (***C***$_{ar}$H), 136.9 (***C***$_{ar}$), 137.8 (***C***$_{ar}$), 154.5 (N(***C***ONPh)$_2$), 154.9 (N***C***(N)N), 172.0 (***C***=O lactam), 172.7 (***C***ON$^{\alpha}$H), 173.1 (***C***OCHFCH$_3$), 173.7 (=NCO(CH$_2$)$_2$***C***O), 174.5 (***C***ONH(CH$_2$)$_2$N<), 174.7 (***C***ON<), 176.4 (=N***C***O(CH$_2$)$_2$CO) ppm; MS (ES, +p) *m/z* (%): 1273 (12) [*M*+H]$^+$, 637 (100) [*M*+2H]$^{2+}$; HRMS (LSI-MS): *m/z* [*M*+H]$^+$ calcd. for $C_{66}H_{87}FN_{13}O_9$: 1272.6581, found: 1272.6562; RP-HPLC: 97 % (t_R = 18.2 min, *k´* = 5.7); $C_{66}H_{86}FN_{13}O_{12}$ (1271.65).

(2*S*)-*N*$^{\omega}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (3.85). A solution of (*S*)-**3.47a** (0.10g, 0.09 mmol) in CH_3CN (6 mL) and 0.1 % aq. TFA (3 mL) was stirred for 2 h at rt. Subsequently, 4 mL of 0.1 % aq. TFA was added and stirring was continued for another 24 h. The deprotection was aborted with a few drops of NEt_3 (pH 9-10) and the solvent was removed under reduced pressure. The aqueous phase was washed

twice with CH_2Cl_2 (20 mL). Then the organic phase was dried over Na_2SO_4, evaporated, and the crude product was purified with the Varian flash chromatography workstation (eluent: CH_2Cl_2 (A), MeOH (B); gradient: 0 to 20 min: A/B 100/0 to 75/25) yielding the title compound as a yellow solid (0.05 g, 60 %). MS (ES, +p) *m/z* (%): 997 (15) $[M+H]^+$, 499 (100) $[M+2H]^{2+}$; $C_{54}H_{65}N_{11}O_8$ (995.50).

Preparation of the N^G-acylated analogs 3.86, 3.87:[21]

3.85 (50 mg, 45 µmol) and NEt_3 (6.2 µL, 45 µmol) was dissolved in CH_3CN (2 mL). The active ester (1.2 eq) was added and the mixture was stirred for 16 h prior to the addition of TFA (1.5 mL). After 2 h the deprotection mixtures were subjected to preparative HPLC to obtain the products as white solids (53-63 %).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-N^{ω}-(propanoyl)argininamide (3.86). Mp 125 °C; ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.12 (t, ^3J = 7.4 Hz, 3H, $COCH_2C\boldsymbol{H}_3$), 1.50 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.50 and 1.70 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.75 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.60 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.19 and 2.26 (m, 4H, 3/5-H piperazine), 2.25 and 2.43 (m, 2H, $C\boldsymbol{H}_2CON^{\alpha}H$), 2.44 (m, 2H, $COC\boldsymbol{H}_2CH_3$), 2.47 (m, 2H, >$NCOC\boldsymbol{H}_2$-cyclopentyl), 3.18 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.43 (br, 4H, 2/6-H piperazine), 3.44 and 3.54 (m, 2H, $NHC\boldsymbol{H}_2CH_2N$<), 3.78 (m, 2H, $NHCH_2C\boldsymbol{H}_2N$<), 4.25 (m, 1H, $C^{\alpha}\boldsymbol{H}$), 4.35 (br, 1H, 11-H dibenzazepine-11-yl), 7.14 (m, 2H, $\boldsymbol{H}_{ar}$), 7.19 (m, 2H, $\boldsymbol{H}_{ar}$), 7.32 (m, 5H, Ph), 7.37 (m, 5H, Ph), 7.40 (m, 1H, $\boldsymbol{H}_{ar}$), 7.44 (m, 1H, $\boldsymbol{H}_{ar}$), 7.52 (m, 1H, $\boldsymbol{H}_{ar}$), 7.83 (d, ^3J = 7.7 Hz, 1H, $\boldsymbol{H}_{ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 8.5 ($COCH_2\boldsymbol{C}H_3$), 24.5 and 24.6 (C-3/4 cyclopentyl), 25.5 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 31.1 ($CO\boldsymbol{C}H_2CH_3$), 38.4 ($NH\boldsymbol{C}H_2CH_2N$<), 39.3 and 39.4 (C-2/5 cyclopentyl), 39.7 (>$NCO\boldsymbol{C}H_2$-cyclopentyl), 41.2 ($NHCH_2\boldsymbol{C}H_2N$<), 42.0 ($\boldsymbol{C}^{\delta}$), 44.7 ($\boldsymbol{C}H_2CON^{\alpha}H$), 45.8 (C-1 cyclopentyl), 47.2 (C-2/6 piperazine), 52.2 and 52.7 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepine-11-yl), 123.0 ($\boldsymbol{C}_{ar}H$), 124.1 (Ph), 126.1 ($\boldsymbol{C}_{ar}H$), 128.1 ($\boldsymbol{C}_{ar}H$), 129.2 ($\boldsymbol{C}_{ar}H$), 129.7 ($\boldsymbol{C}_{ar}H$), 130.2 (Ph), 131.3 ($\boldsymbol{C}_{ar}H$), 131.9 ($\boldsymbol{C}_{ar}H$), 132.4 ($\boldsymbol{C}_{ar}$), 133.2 ($\boldsymbol{C}_{ar}H$), 136.9 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 ($N(\boldsymbol{C}ONPh)_2$), 155.0 ($N\boldsymbol{C}(N)N$), 171.7 ($\boldsymbol{C}$=O lactam), 172.8 ($\boldsymbol{C}ON$<), 174.5 ($\boldsymbol{C}ONH(CH_2)_2N$<), 174.7 ($\boldsymbol{C}ON^{\alpha}H$), 177.7 ($\boldsymbol{C}OCH_2CH_3$) ppm; MS (ES, +p) *m/z* (%): 477 (100) $[M+2H]^{2+}$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{52}H_{62}N_{11}O_7$: 952.4834, found: 952.4845; RP-HPLC: 99 % (t_R = 13.5 min, k' = 4.0); $C_{52}H_{61}N_{11}O_7$ (951.48).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-(4-fluorobenzoyl)-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (3.87). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.51 and 1.79 (m, 2H, $C^{\beta}\boldsymbol{H}_2$), 1.50 and 1.64 (m, 4H, 2/5-H cyclopentyl), 1.51 and 1.63 (m, 4H, 3/4-H cyclopentyl), 1.66 (m, 2H, $C^{\gamma}\boldsymbol{H}_2$), 2.12 and 2.18 (m, 4H, 3/5-H piperazine), 3.26 (m, 2H, $C^{\delta}\boldsymbol{H}_2$), 3.39 and 3.46 (m, 4H, 2/6-H piperazine), 3.54 (m, 2H, NHC$\boldsymbol{H}_2$CH$_2$N<), 3.78 (m, 2H, NHCH$_2$C$\boldsymbol{H}_2$N<), 4.24 (br, 1H, 11-H dibenzazepine-11-yl), 4.28 (m, 1H, $C^{\alpha}\boldsymbol{H}_2$), 7.10 (m, 1H, $\boldsymbol{H}_{ar}$), 7.12 (m, 1H, $\boldsymbol{H}_{ar}$), 7.18 (m, 2H, Ph), 7.28 (m, 1H, $\boldsymbol{H}_{ar}$), 7.30 (m, 8H, Ph), 7.37 (m, 4H, Ph), 7.41 (m, 1H, $\boldsymbol{H}_{ar}$), 7.49 (m, 1H, $\boldsymbol{H}_{ar}$), 7.80 (m, 1H, $\boldsymbol{H}_{ar}$), 8.00 (m, 2H, Ph) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 24.5 and 24.6 (C-3/4 cyclopentyl), 25.4 ($\boldsymbol{C}^{\gamma}$), 30.0 ($\boldsymbol{C}^{\beta}$), 38.5 (NH$\boldsymbol{C}$H$_2$CH$_2$N<), 39.3 (C-2/5 cyclopentyl), 39.4 and 39.6 (>NCO$\boldsymbol{C}$H$_2$-cyclopentyl), 41.2 (NHCH$_2\boldsymbol{C}$H$_2$N<), 42.3 ($\boldsymbol{C}^{\delta}$), 42.6 and 47.6 (C-2/6 piperazine), 44.7 ($\boldsymbol{C}$H$_2$CON$^{\alpha}$H), 45.9 (C-1 cyclopentyl), 52.1 and 52.6 (C-3/5 piperazine), 53.7 ($\boldsymbol{C}^{\alpha}$), 75.8 (C-11 dibenzazepin-11-yl), 117.2 ($\boldsymbol{C}_{ar}$H), 117.3 ($\boldsymbol{C}_{ar}$H), 123.0 ($\boldsymbol{C}_{ar}$H), 124.1 (Ph), 126.0 ($\boldsymbol{C}_{ar}$H), 128.1 ($\boldsymbol{C}_{ar}$H), 128.9 ($\boldsymbol{C}_{ar}$H), 129.4 ($\boldsymbol{C}_{ar}$H), 129.9 ($\boldsymbol{C}_{ar}$), 130.2 (Ph), 131.1 ($\boldsymbol{C}_{ar}$H), 131.6 ($\boldsymbol{C}_{ar}$H), 132.2 (Ph), 132.4 ($\boldsymbol{C}_{ar}$), 133.1 ($\boldsymbol{C}_{ar}$H), 136.8 ($\boldsymbol{C}_{ar}$), 137.8 ($\boldsymbol{C}_{ar}$), 154.5 (N($\boldsymbol{C}$ONPh)$_2$), 155.5 (NC(N)N), 168.6 ($\boldsymbol{C}$OC$_6$H$_4$F), 172.1 ($\boldsymbol{C}$=O lactam), 172.7 ($\boldsymbol{C}$ON$^{\alpha}$H), 174.5 ($\boldsymbol{C}$ONHC$_2$H$_4$N<), 174.7 ($\boldsymbol{C}$ON<) ppm; MS (ES, +p) *m/z* (%):1018 (100) $[M+H]^+$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{56}H_{61}FN_{11}O_7$: 1018.4739, found: 1018.4742; RP-HPLC: 98 % (t_R = 21.5 min, k' = 7.0); $C_{56}H_{60}FN_{11}O_7$ (1017.47).

3.5.3 Investigation of the Chemical Stability

The stability of the Y_2R antagonists (*S*)-**3.48**-(*S*)-**3.50**, (*S*)-**3.53**-(*S*)-**3.55**, (*S*)-**3.57**, (*S*)-**3.58**, (*S*)-**3.63**, (*S*)-**3.69**, (*S*)-**3.72** and (*S*)-**3.74** regarding the formation of BIIE 0246 ((*S*)-**3.47**) was investigated at neutral pH in FACS-buffer (cf. section 3.5.1). Incubation was started by addition of 10 µL of a 1 mM solution of the compounds in DMSO to 190 µL of buffer to give a final concentration of 50 µM. After incubation times between 15 and 180 min a 80 µL aliquot was taken and diluted with a mixture of acetonitrile, H_2O and 1 % aq. TFA (5:1:4, 80 µL). 100 µL of the resulting solution (pH = 2-3) was analyzed by HPLC on a RP-column (Eurospher-100 C18, 250 × 4 mm, 5 µm, Knauer, Berlin, Germany) using a system from Thermo Separation Products (composed of a SN400 controller, a P4000 pump, a degasser (Degassex DG-4400, phenomenex), an AS3000 autosampler and a Spectra Focus UV-VIS detector. Mixtures of acetonitrile (A) and 0.05 % aq. TFA (B) were used as mobile phase (gradient: 0-30 min: A/B 20/80 to 95/5, 30 to 37 min: A/B 95/5). The flow rate was set to 0.8 mL/min, the column temperature to 30 °C and the detection wavelength to 220 nm. The quantity of decomposition product (*S*)-**3.47** was estimated from the peak integrals of the incubation sample and a reference chromatogram of the analyzed compound.

3.5.4 Pharmacology: Cell Culture, Fura-2 Assay and Flow Cytometry

Cell culture. CHO cells were cultured as described elsewhere.[29]

Fura-2 assay on CHO cells. The fura-2 assay was performed as previously described[30] using a LS50 B spectrofluorimeter from Perkin Elmer (Überlingen, Germany).

Flow cytometric competition binding assay. The binding assay was performed as described elsewhere[29] with the following adaptations. Samples were measured without further processing using a FACSCalibur™ flow cytometer from Becton Dickinson (Heidelberg, Germany), equipped with an argon laser (488 nm) and a red diode laser (635 nm); instrument settings were: FSC: E-1, SSC: 350 V, FL4: 800 V, Flow: HI; measurement stopped when 10000 gated events were counted. The experiments were performed using 490 µL of cell suspension, 5 µL of Cy5-pNPY (final concentration 5 nM) and 5 µL of test compound (100-fold concentrated). If indicated, 5 µL of Dy-635-pNPY (final concentration 10 nM) was used as fluorescent ligand. Incubation time was 90 min at room temperature. K_i values were obtained from 2-3 independent experiments.

Flow cytometric selectivity assay. The selectivity of the new Y_2R antagonists for human NPY Y_2 over Y_1, Y_4 and Y_5 receptors was proven by flow cytometric binding assays on Cy5-pNPY (Y_1R, Y_5R), and Cy5-[K^4]-hPP (Y_4R) according to previously described methods.[29, 44-45] All flow cytometric measurements were performed on a FACSCalibur™ flow cytometer from Beckton Dickinson (Heidelberg, Germany), equipped with an argon laser (488 nm) and a red diode laser (635 nm). The compounds were tested at two concentrations (1 µM and 10 µM) in duplicates.

3.6 References

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Chapter 4 Bivalent Argininamide-Type NPY Y_2 Receptor Antagonists

4.1 Introduction

For many years G protein-coupled receptors (GPCRs) were thought to exist and function exclusively as monomers. However, there is a growing number of reports that suggest homo- and hetero-dimerization of numerous GPCRs, e.g. adrenergic receptors,[1] $GABA_B$ receptors,[2-3] opioid receptors,[4-6] muscarinic receptors,[7-9] vasopressin receptors,[10-11] chemokine receptors,[12-13] dopamine receptors,[14-15] histamine receptors,[16-17] etc.[18-21] FRET experiments clearly proved the ability of human neuropeptide Y receptors Y_1, Y_2 and Y_5 (hY_1R, hY_2R, hY_5R) to form homo-dimers, whereas the hY_2R is less prone to dimerization than the hY_1R and the hY_5R, respectively.[22] More detailed investigations on the localization, formation and composition of Y_2R dimers were performed by Parker *et al*. on different tissues of the rabbit.[23-24] Therein, the Y_2R was observed to be largely dimeric in the kidney, but monomeric in the forebrain, possibly due to variable levels of G-proteins containing the $G\alpha_i$ subunit in different tissues.[24] Furthermore, in the renal cortex a protein-complex of about 300 kDa was detected, which was postulated to result from the association of both Y_2R protomers of the homo-dimer with two G-protein αβγ hetero-trimers.[23] The dimerization might be enabled by a tryptophan-tryptophan H-bonding in TM4,[24] as suggested for dopamine D_2 receptor dimers.[25] Commonly applied techniques for the identification of GPCR oligomers include co-immuno-precipitation,[26] functional studies (e.g. measurement of GPCR-β-arrestin interactions with β-galactosidase enzyme complementation technique[27]), mutation studies of putative dimerization motives,[28] bioluminescence resonance energy transfer (BRET)[1] and molecular modeling.[29] However, all experiments demonstrating a physical interaction between GPCRs have up to now been performed *ex vivo* in transfected cells, which implies non-physiological cellular environments and – in most cases – unrealistically high concentrations of receptors in the membranes. In this context, the *in vivo* demonstration of GPCR dimers of the luteinizing hormone receptor (LHR) by Rivero-Müller *et al.* constitutes a major contribution to the proof of GPCR oligo-merization.[30] Co-transfection with a binding-deficient and a signaling-deficient LHR resulted in a quasi wild-type phenotype after activation with LH. Thus, functional activity could only be generated by the dimerization of the loss-of-function LHR mutants.[31]

The use of bivalent ligands as an alternative to the aforementioned methods offers the possibility to investigate native GPCRs, expressed endogenously by normal wild-type cells. Such compounds, defined as two pharmacophoric moieties linked through a spacer, should bring more insight into the phenomenon of GPCR dimerization. Moreover, the bivalent ligand approach is discussed as an alternative drug design strategy. On the one hand, bivalent ligands can interact with the orthosteric and an additional allosteric binding site (cf. Figure 4.1). Such key interactions may be formed with residues that reside within extracellular loop domains,[33] which often display lower homology between receptor subtypes in comparison to transmembrane domains. Thus, a higher degree of receptor-subtype selectivity may be achieved by this approach. On the other hand, bivalent ligands addressing two protomers should enhance tissue specificity, as receptor oligomers (especially hetero-oligomers) are co-expressed in certain tissues with a distinct pharmacology and function.[27] Hence, concerning side-effects, ligands combining two distinct pharmacophoric moieties in one molecule should be superior to combined administration of two monomeric drugs. This renders the bivalent ligand approach a very interesting topic in drug design.

However, there are a few spatial requirements to be fulfilled for a successful application of bivalent ligands: firstly, the spacer length must be sufficient to address the binding sites of both protomers. Secondly, flexibility and appropriate chemical nature of the linker are essential for the correct positioning of each pharmacophore. Studies on bivalent based drug design applied to opioid and serotonin receptors suggested a distance between the binding sites of 22-32 Å (corresponding to 16-24 atoms).[32, 34-35]

The distance between the two binding sites is difficult to predict as there are several variants of contact dimers discussed in the literature.[25, 32, 34] Molecular modeling studies revealed a distance of about 27 Å in case of a TM5,6 interface, while a TM4,5 interface leads to a substantially longer distance of approximately 32 Å.[32] Also, the linker might span the transmembrane domains or it may bridge the two binding sites on the extracellular side, complicating the prediction of an optimal spacer length. Moreover, an affinity-enhancing effect might occur due to cooperativity by interaction with an allosteric site, rather than bridging the orthosteric pockets of two protomers (Figure 4.1). Thus, length and chemical nature of the spacer must be optimized for each GPCR dimer empirically

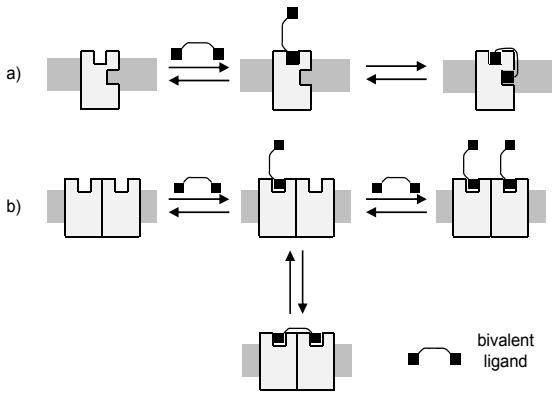


Figure 4.1. Illustration of the bivalent ligand concept for bridging of a hypothetical GPCR dimer. a) Occupation of an allosteric binding site by the second pharmacophore of a bivalent ligand. b) The unoccupied dimer undergoes univalent binding followed by either bridging of the bivalent ligand, or binding of a second ligand to give the dimer with both sides occupied. Adopted from Portoghese.[32]

by screening several ligands.[36] To date, besides the aforementioned opioid and serotonin receptors, a variety of successful applications of the bivalent ligand approach to GPCRs is reported in the literature.[36-39] In case of NPY receptors, the first studies focused on the preparation of peptidic bivalent antagonists derived from porcine NPY (pNPY). The first dimeric ligands, consisting of two nonapeptides mimicking the C-terminal part, were bridged by either disulfide bonds, 2,6-diaminopimelic acid and 2,3-diaminopropionic acid, respectively. These antagonists showed high affinity for both the Y_1R and the Y_2R (K_i (Y_1R) = 0.2-2.9 nM; K_i (Y_2R) = 0.02-8.8 nM).[40] Balasubramaniam *et al.* constructed the first highly potent and selective Y_1R bivalent antagonist based on the C-terminal hexapeptide of NPY.[41] The only non-peptidic bivalent ligands for the Y_1R known so far were prepared in our laboratory as analogs of BIBP 3226[42] linked by a spacer attached to the guanidino group.[43]

This chapter presents the first non-peptide bivalent Y_2R antagonists, derived from BIIE 0246[44] by application of the guanidine-acylguanidine bioisosteric approach in analogy to the recently developed Y_1R bivalent ligands. The amine precursors used for the linkage with dicarboxylic acids are presented in Figure 4.2.

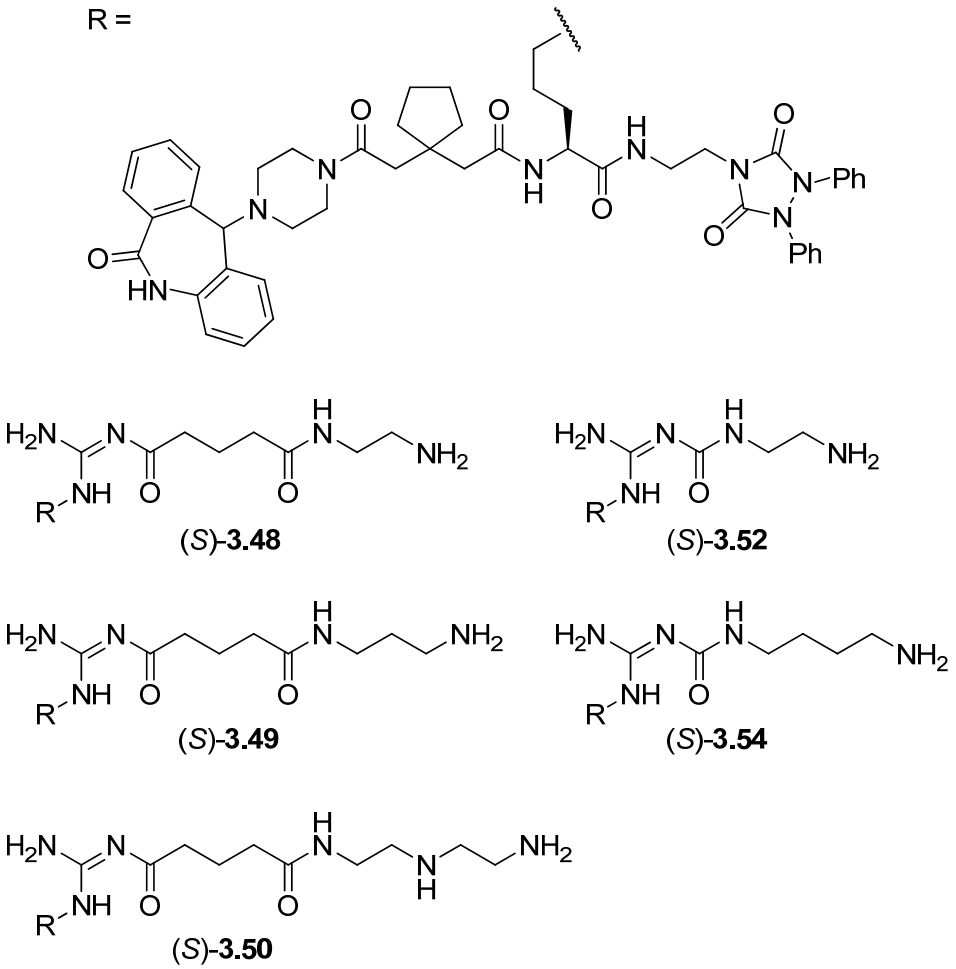
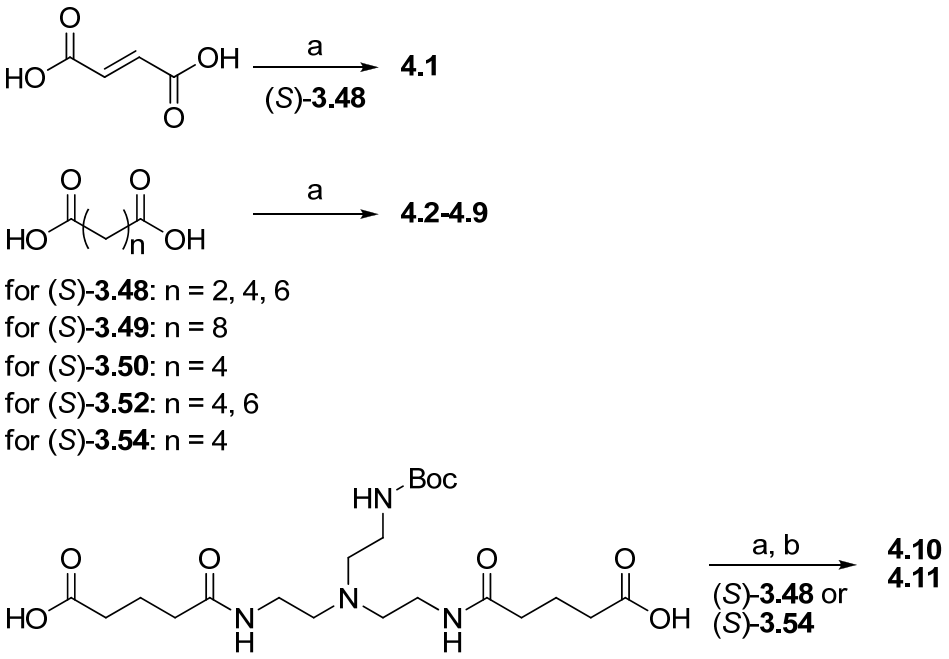


Figure 4.2. Argininamide-type amine precursors derived from BIIE 0246 used for the preparation of bivalent Y_2R antagonists.

4.2 Chemistry

The synthesis of the amine precursors ((*S*)-**3.48**-(*S*)-**3.50**, (*S*)-**3.52** and (*S*)-**3.54**), which were used for the preparation of bivalent Y_2R antagonists is described in chapter 3 (for structures cf. Figure 4.2). These building blocks contain two amino groups prone to acylation, namely the free $N^{\omega'}H_2$ and the terminal amine in the acyl- or carbamoyl linker. The terminal amino function is more reactive than the acylguanidine due to higher basicity and less sterical hindrance. Hence, the guanidino function was not protected as the synthetic strategy aimed at a minimum number of reaction steps and maximal purity on the low mg scale rather than at optimization of yields and synthetic routes. Various alkanedioic acids and amine precursors, respectively, were employed to synthesize bivalent ligands with spacer lengths between 16 and 35 atoms linking the two pharmacophoric moieties. Furthermore, the aforementioned building blocks (Figure 4.2) were chosen for linkage in order to obtain compounds differing in the chemical nature of the spacer.

For the preparation of the bivalent ligands **4.1**-**4.9** the amine precursors were acylated with the corresponding alkanedioic acid in an EDC/DMAP-mediated coupling step under microwave irradiation followed by purification with preparative HPLC (Scheme 4.1; for structures of the bivalent ligands **4.1**-**4.11** cf. Table 4.1). In case of the antagonists **4.10** and **4.11**, the terminal amino group of the branched linker was Boc-protected to avoid side reactions. Thus, acidic deprotection was necessary prior to the purification of the product (Scheme 4.1). The branched linkers were kindly provided by Dr. M. Keller from our laboratory.[43]



Scheme 4.1. Synthesis of the bivalent ligands **4.1**-**4.11**. Reagents and conditions: a) alkanedioic acid (1 eq), amine precursor (2.4 eq), EDC × HCl (2.1 eq), NEt_3 (6 eq), DMAP (cat.), CH_2Cl_2, DMF, MW, 35 min, 65 °C; b) TFA/CH_2Cl_2 1:1 (v/v), 2 h, rt.

The title compounds were obtained in low yields (10-30 %). HPLC reaction monitoring revealed four major peaks (Figure 4.3 shows a representative chromatogram for the synthesis of **4.6**). After HPLC purification several compounds were identified by MS analysis. Peak A corresponds to non-reacted amine precursor which was used in excess. Peak B is assigned to a trifluoroacetylated by-product. This side reaction resulted from the application of the amine precursors as TFA salts. As these salts proved to be highly stable, they were not converted into the less stable free amines before coupling (see also Chapter 3.2.2). The monoacylated compound corresponds to peak C, whereas peak D with the longest retention time was identified as the respective bivalent ligand.

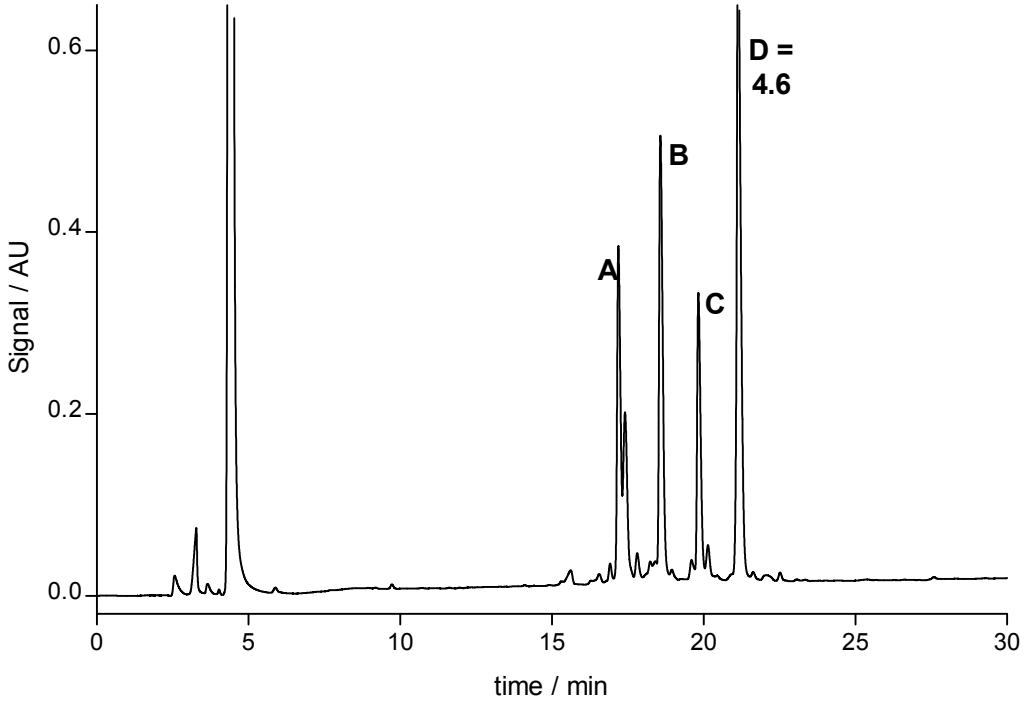
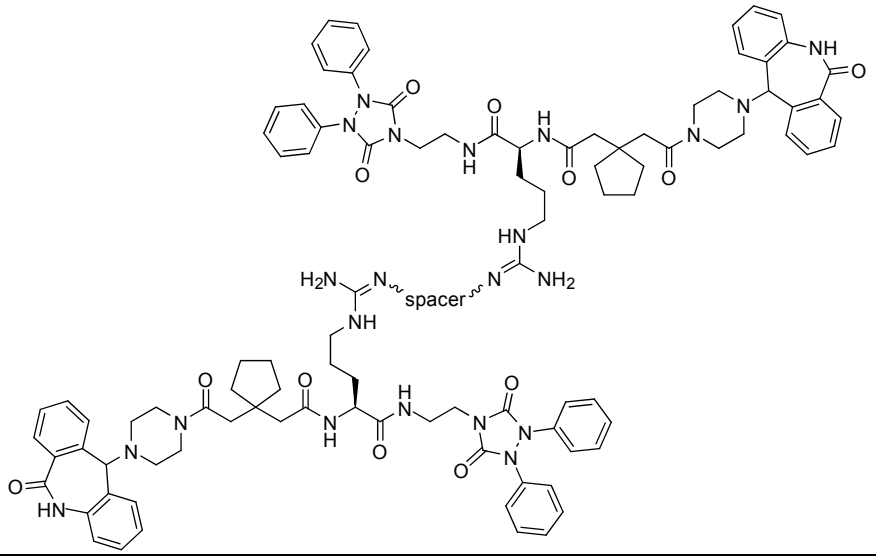


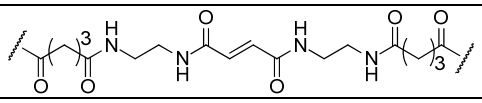
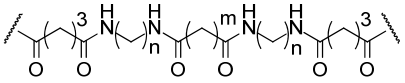
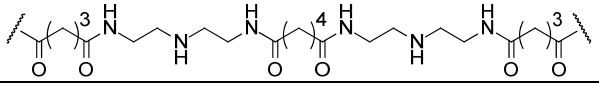
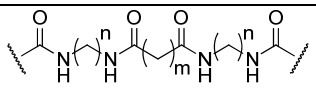
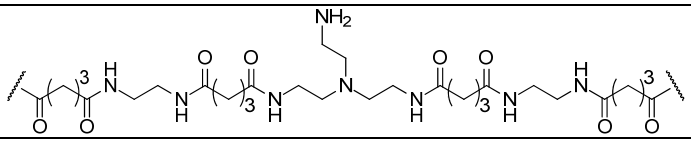
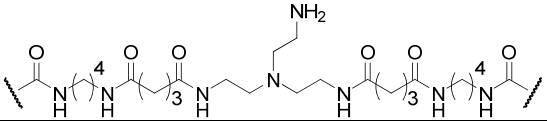
Figure 4.3. HPLC reaction monitoring of the preparation of bivalent ligands exemplified by the synthesis of **4.6**. Conditions: eluent: mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5, 30 to 40 min: 95/5. Chromatogram cut after 30 min as there were no further peaks. Peak A: starting material (*S*)-**3.50** (t_R = 17.2 min), peak B: TFA-acylated compound (t_R = 18.6 min), peak C: monoacylated by-product (t_R = 19.8 min), peak D: product **4.6** (t_R = 19.8 min).

4.3 Pharmacological Results and Discussion

The synthesized bivalent ligands were investigated for Y_2R binding in a flow cytometric binding assay using CHO cells, stably expressing the hY_2R,[45] and fluorescence-labeled Cy5-pNPY. NPY Y_2R antagonistic activities were determined in a spectrofluorimetric Ca^{2+} assay (fura-2 assay) on CHO cells, stably expressing the hY_2R.[46] The selectivity for the Y_2R over Y_1R, Y_4R and Y_5R was examined by means of flow cytometry.[47]

Table 4.1. Structures, Y_2R antagonistic activities (K_B) and binding affinities (K_i) of the bivalent Y_2R antagonists.



No	Spacer	No of atoms[a]	K_B / nM[b]	K_i / nM[c]
4.1		22	82 ± 2	161 ± 10
				
	n = 2, m = 2	22	5 ± 3	69 ± 7
4.3	n = 2, m = 4	24	61 ± 2	103 ± 57
4.4	n = 2, m = 6	26	44 ± 27	300 ± 81
4.5	n = 3, m = 8	30	241 ± 68	61 ± 4
4.6		30	15 ± 3	21 ± 6
				
	n = 2, m = 4	16	4.5 ± 1.9	74 ± 13
4.8	n = 2, m = 6	18	119 ± 41	83 ± 7
4.9	n = 4, m = 4	20	30 ± 12	171 ± 18
4.10		35	11 ± 2	62 ± 34
4.11		31	20 ± 4	97 ± 15
(*S*)-**3.47**[d]	--	--	5.6 ± 0.4	10.2 ± 1.1

[a] Number of atoms between the two guanidine functions ("length" of the spacer). [b] Inhibition of 70 nM pNPY-induced $[Ca^{2+}]_i$ mobilization in CHO cells; mean values ± SEM (n = 2-3). [c] Flow cytometric binding assay using Cy5-pNPY (K_D = 5.2 nM; c = 5.0 nM) in CHO cells; mean values ± SEM (n = 2-3). [d] BIIE 0246.

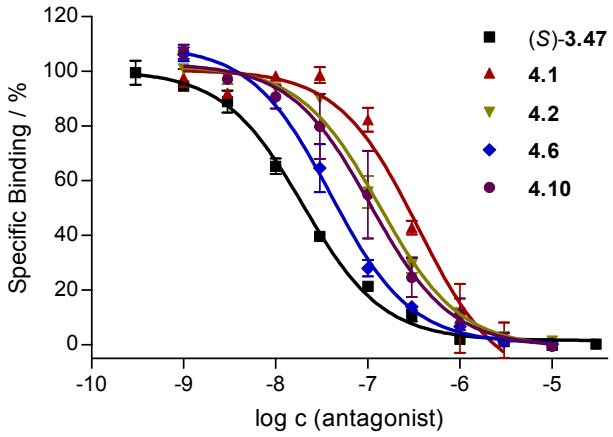


Figure 4.4. Displacement of 5 nM Cy5-pNPY by (*S*)-**3.47** (BIIE 0246) and the acylguanidine-type bivalent ligands **4.1**, **4.2**, **4.6**, **4.10**, respectively (mean values ± SEM; n = 2).

Functional and binding data as well as the structures of the prepared bivalent compounds are summarized in Table 4.1. All bivalent NPY Y_2R antagonists showed binding affinities in the two to three-digit nM range. In most cases, the K_B values determined in the calcium assay were even lower. Rigidization by introduction of a double-bond in the spacer is moderately tolerated by the receptor as shown for compound **4.1** in comparison with the flexible analog **4.2** (K_i (**4.1**) = 161 nM vs. K_i (**4.2**) = 69 nM; Figure 4.4). The acylguanidine-bridged ligands **4.2**-**4.5** showed the highest affinities with linker lengths of 22 and 30 atoms, respectively, bridging the two pharmacophoric moieties (K_i (**4.2**) = 61 nM; K_i (**4.5**) = 69 nM), whereas Y_2R antagonistic activities decreased with increasing spacer lengths. The introduction of a second positively charged amino group in the linker of antagonist **4.6** led to a gain in affinity. With a K_i value of 21 nM **4.6** (spacer length: 30 atoms) was the most potent bivalent ligand of this small series (Figure 4.4), confirming a possible additional electrostatic interaction with the receptor, as already discussed for the monovalent ligands (cf. Chapter 3).

The affinity of carbamoylguanidine **4.9** (spacer length: 20 atoms) corresponds to about half of that of the lower homologs **4.7** and **4.8** with spacer lengths of 16 and 18 atoms, respectively. The K_i values of the latter are in the same range of those of **4.2** and **4.5**. Unfortunately, acylguanidine analogs of **4.7**-**4.9** have not been available, yet, in order to compare the impact of alkanoyl- and carbamoyl-linkage on binding. As already observed for argininamide-type Y_2R antagonists (cf. Chapter 3), the presence of the carbamoyl NH-group might implicate a different orientation of the N^G-substituent. Moreover, carbamoylguanidines are slightly more basic than acylguanidines. This should be affinity-enhancing, as the guanidino group is supposed to undergo an ionic interaction with an acidic residue of the Y_2R.

All bivalent Y_2R ligands show mono-phasic competition binding curves with Hill slopes not statistically significantly different from unity. Furthermore, the binding affinities of the bivalent antagonists are lower than that of the parent compound BIIE 0246. Thus, bridging of a Y_2R dimer, or an allosteric modulation by these bivalent ligands is

very unlikely. Nevertheless, a larger library of structurally diverse bivalent Y_2R ligands is necessary for more detailed structure-activity investigations.

The bivalent ligands **4.10** and **4.11** with a terminal positively charged amino function in the branched part of the linker revealed K_i values below 100 nM. These compounds are considered appropriate precursors for the synthesis of radio- or fluorescence-labeled bivalent Y_2R antagonists.

The introduction of a second pharmacophoric entity into the bivalent ligands might change the selectivity profile, as observed for several argininamide-type bivalent Y_1R antagonists. A few eutomeric (*R*,*R*)-configured compounds appeared to be highly potent ligands[43] with a decreased selectivity against Y_4R, and some of the distomeric (*S*,*S*)-configured antagonists[43] turned out to bind at the Y_4R, rather than at the Y_1R.[48] Thus, the high selectivity of monovalent antagonists used as building blocks is not necessarily retained with the corresponding bivalent ligands. Nevertheless, in the case of the bivalent Y_2R antagonists presented in this thesis, the Y_2R selectivity was not compromised compared to the monovalent parent compounds, as confirmed by flow cytometric binding studies (Table 4.2).

Table 4.2. NPY receptor subtype selectivity of the bivalent Y_2R antagonists.

No	K_i / nM[a]			No	K_i / nM[a]		
	Y_1R	Y_4R	Y_5R		Y_1R	Y_4R	Y_5R
4.1	> 3500	> 6500	> 5000	**4.7**	> 3500	> 6500	> 5000
4.2	> 3500	> 6500	> 5000	**4.8**	> 3500	> 6500	> 5000
4.3	> 3500	> 6500	> 5000	**4.9**	> 2000	> 6500	> 5000
4.4	> 3500	> 6500	> 5000	**4.10**	> 1500	> 3200	> 5000
4.5	> 3500	> 6500	> 5000	**4.11**	> 800	> 7500	> 5000
4.6	> 2000	> 650	> 5000				

[a] Flow cytometric binding assays using 10 nM Cy5-pNPY (Y_1R), 5 nM Cy5-pNPY (Y_5R) or 3 nM Cy5-[K^4]-hPP (Y_4R) in HEL-Y_1 cells, CHO-Y_4 cells, and HEC-1B-Y_5 cells, respectively.

4.4 Summary and Conclusion

A small set of bivalent argininamide-type Y_2R antagonists was synthesized by linkage of various monomeric amine precursors derived from the reference compound BIIE 0246, using aliphatic dicarboxylic acids or a branched dicarboxylic acid comprising a primary amine. These bivalent ligands cover a wide range of linker lengths (number of atoms: 16-35). Flow cytometric determination of binding data revealed high Y_2R affinities (K_i values in the range of 61 to 300 nM) and high selectivity for the Y_2R compared to Y_1R, Y_4R and Y_5R. Interestingly, compound **4.6**, bearing two additional positively charged amines in the acyl linker was the only compound that exhibited a binding affinity (K_i = 21 nM) and potency (K_B = 15 nM) in the same range as the corresponding monovalent N^G-(ω-aminoalkylacylated) and N^G-(ω-aminoalkylcarbamoylated) antagonists. This is in agreement with the affinity-enhancing effect of a positively charged amino group in the N^G-substituent of monovalent acylguanidine-type Y_2R antagonists (cf. Chapter 3), suggesting an additional electrostatic interaction with the receptor. The prepared bivalent ligands exhibited monophasic competition binding curves with Hill slopes close to unity and decreased binding affinities compared to the monovalent analogs. Hence, positive cooperativity due to interactions with an allosteric site, or even by bridging two monomers of the Y_2R could not be observed.

Lastly, the identification of receptor oligomers by non-labeled bivalent ligands is very challenging as there are only competition binding data available. Thus, a larger library of compounds and more detailed pharmacological studies would be indispensable to draw reliable conclusions from competition binding curves (bi-phasic curves, Hill slope with statistically high significance). Therefore, radio- and fluorescent-labeled bivalent ligands could serve as pharmacological tools for the identification and investigation of putative receptor oligomers. In particular, radiolabeled bivalent ligands could be useful to determine the ligand-receptor stoichiometry in order to distinguish between binding to monomeric or dimeric Y_2Rs. The compounds **4.10** and **4.11** can be regarded the first amine precursors allowing for the synthesis of labeled ”twin compounds”. Of course, additional branched analogs with various linker lengths have to be prepared and checked for stability under assay conditions in order to select an appropriate candidate for labeling.

4.5 Experimental Section

4.5.1 General Experimental Conditions

Unless otherwise stated, chemicals and solvents were purchased from commercial suppliers and used without further purification. The dicarboxylic acids were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany) and from Merck KGaA (Darmstadt, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under argon atmosphere; DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Thin layer chromatography was performed on Merck silica gel 60 F_{254} TLC aluminum plates. Spots were visualized with UV light at 254 nm, and/or ninhydrin or ammonium molybdate/cerium (IV) sulfate solution.

NMR spectra were recorded on an Avance 300, an Avance 400 and an Avance III 600 spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany) with TMS as external standard. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), quat (quartet), q (quintet), m (multiplet), br (for broad singulet), as well as combinations thereof. Low resolution mass spectrometry analysis (MS) was performed in-house on a Finnigan ThermoQuest TSQ 7000 (ES-MS) and a Finnigan SSQ 710A (EI-MS 70 eV, CI-MS). High resolution mass spectrometry was performed on a Finnigan MAT 95 (LSI-MS). Lyophilization was done with a Christ alpha 2-4 LD equipped with a vacuubrand RZ 6 rotary vane vacuum pump. Preparation of the bivalent ligands was performed on the Microwave Synthesizer Initiator from Biotage (Uppsala, Sweden).

Preparative HPLC was performed with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector and a RP-column (Nucleodur 100-5 C18ec, 250 × 21 mm, 5 µm) at a flow rate of 22 mL/min. Mixtures of acetonitrile and 0.1 % aq. TFA were used as mobile phase. Acetonitrile was removed from the eluates under reduced pressure (final pressure: 90 mbar) at 45 °C prior to lyophilization. Analytical HPLC analysis was performed on two different systems (Thermo Separation Products, Merck) which are listed in detail below. Mixtures of acetonitrile (A) and 0.05 % aq. TFA (B) (Thermo Separation Products), and mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B) (Merck) respectively were used as mobile phase. Helium degassing was used throughout.

The buffer for the Y_2R binding studies on CHO cells was prepared by the addition of BSA (1 %) and bacitracin (100 µg/mL) to a buffer (pH 7.4) consisting of HEPES (25 mM), $CaCl_2 \times 2\ H_2O$ (2.5 mM), $MgCl_2 \times 6\ H_2O$ (1 mM). The loading buffer (pH 7.4) for the determination of the mobilization of intracellular Ca^{2+} in CHO cells was prepared by dissolving NaCl (120 mM), KCl (5 mM), $MgCl_2 \times 6\ H_2O$ (2 mM), $CaCl_2 \times 2\ H_2O$ (1.5 mM), HEPES (25 mM), and glucose (10 mM). Stock solutions of all

compounds were prepared in DMSO at concentrations of 2 or 10 mM and stored at –20 °C.

Analytical HPLC systems:
System 1: Thermo Separation Products; composed of a SN400 controller, a P4000 pump, a degasser (Degassex DG-4400, phenomenex), an AS3000 autosampler and a Spectra Focus UV-VIS detector; flow rate: 0.8 mL/min; UV detection: 220 nm.
System 2: Merck; composed of a L-5000 controller, a 655A-12 pump, a 655A-40 autosampler and a L-4250 UV-VIS detector; flow rate: 0.7 mL/min; UV detection: 220 nm.

Applied gradients:
Gradient 1 (Thermo Separation Products): 0 to 30 min: A/B 20/80 to 95/5, 30 to 37 min: 95/5.
Gradient 2 (Merck): 0 to 30 min: A/B 20/80 to 95/5, 30 to 40 min: 95/5.
The purity of all compounds was determined with HPLC system 1 (gradient 1) on a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany) for compounds **4.1**-**4.4**, **4.6**, **4.9**-**4.11** and a Nucleodur H-Tec 100-5 C18 column (250 × 4 mm, 5 µm, Macherey-Nagel, Düren, Germany) for compounds **4.5**, **4.8**, **4.9** respectively. The HPLC system 2 (gradient 2) was used for reaction monitoring with a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany).

4.5.2 Chemistry: Experimental Protocols and Analytical Data

General procedure for the preparation of the bivalent ligands 4.1-4.11:

NEt_3 (6 eq) and DMAP (0.1 eq) were added to a stirred solution of the pertinent amine precursor (2.4 eq) and the corresponding alkanedioic acid (1 eq) in CH_2Cl_2. EDC × HCl (2.1 eq) was added and the mixture was stirred in a microwave synthesizer for 35 min at 65 °C. The reaction was stopped by addition of 10 % aq. TFA (corresponding to 2-3 eq of TFA) and the solvents were removed under reduced pressure. Purification with preparative HPLC and lyophilization afforded the products as white fluffy solids in yields from 10 to 30 %.

(11*E*)-N^1,N^{22}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]-azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,10,13,18-tetroxo-6,9,14,17-tetraazadocosa-11-enediamide (4.1). MS (ES, +p) *m/z* (%): 1093 (40) $[M+2H]^{2+}$, 729 (100) $[M+3H]^{3+}$; RP-HPLC: 99 % (t_R = 20.1 min, k' = 6.4); $C_{116}H_{138}N_{26}O_{18}$ (2138.07).

***N*^{1},*N*^{22}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-amino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,10,13,18-tetroxo-6,9,14,17-tetraazadocosanediamide (4.2).** ^{1}H NMR (300 MHz, CD_3OD): δ = 1.42-1.80 (m, 24H, 2 x 3/4-H cyclopentyl, 2 x 2/5-H cyclopentyl, 2 x $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$), 1.93 (m, 4H, 2 x $COCH_2C\boldsymbol{H}_2CH_2CO$), 2.00-2.30 (m, 16H, 2 x 3/5-H piperazine, 2 x $COC\boldsymbol{H}_2CH_2C\boldsymbol{H}_2CO$), 2.28 and 2.46 (m, 4H, 2 x $C\boldsymbol{H}_2CON^{\alpha}H$), 2.46 (m, 8H, $CO(C\boldsymbol{H}_2)_2CO$, 2 x >$NCOC\boldsymbol{H}_2$-cyclopentyl), 3.18 (m, 4H, 2 x $C^{\delta}\boldsymbol{H}_2$), 3.26 (m, 8H, 2 x $NH(C\boldsymbol{H}_2)_2NH$), 3.35-3.57 (m, 12H, 2 x 2/6-H piperazine, 2 x $NHC\boldsymbol{H}_2CH_2N$<), 3.78 (br, 4H, 2 x $NHCH_2C\boldsymbol{H}_2N$<), 4.25 (br, 4H, 2 x 11-H dibenzazepine-11-yl, 2 x $C^{\alpha}\boldsymbol{H}$), 7.13 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.20 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.36 (m, 20H, Ph), 7.36 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.42 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.51 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.80 (m, 2H, $\boldsymbol{H}_{Ar}$) ppm; MS (ES, +p) *m/z* (%): 1094 (10) $[M+2H]^{2+}$, 730 (75) $[M+3H]^{3+}$, 548 (100) $[M+4H]^{4+}$; RP-HPLC: 97 % (t_R = 19.6 min, *k´* = 6.3); $C_{116}H_{140}N_{26}O_{18}$ (2185.08).

***N*^{1},*N*^{24}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-amino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,10,15,20-tetroxo-6,9,16,19-tetraazatetracosanediamide (4.3).** MS (ES, +p) *m/z* (%): 1108 (20) $[M+2H]^{2+}$, 739 (80) $[M+3H]^{3+}$, 555 (100) $[M+4H]^{4+}$; RP-HPLC: 97 % (t_R = 19.5 min, *k´* = 6.2); $C_{118}H_{144}N_{26}O_{18}$ (2213.12).

***N*^{1},*N*^{26}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-amino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,10,17,22-tetroxo-6,9,18,21-tetraazahexacosanediamide (4.4).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.31 (m, 4H, $CO(CH_2)_2(C\boldsymbol{H}_2)_2(CH_2)_2CO$), 1.49-1.80 (m, 28H, 2 x 3/4-H cyclopentyl, 2 x 2/5-H cyclopentyl, 2 x $C^{\beta}\boldsymbol{H}_2C^{\gamma}\boldsymbol{H}_2$, $COCH_2C\boldsymbol{H}_2(CH_2)_2C\boldsymbol{H}_2CH_2CO$), 1.91 (p, ^{3}J = 7.3 Hz, 4H, 2 x $COCH_2C\boldsymbol{H}_2CH_2CO$), 2.02-2.13 (m, 8H, 2 x 3/5-H piperazine), 2.16 and 2.24 (2 x m, 8H, 2 x $COC\boldsymbol{H}_2CH_2C\boldsymbol{H}_2CO$), 2.16 and 2.46 (m, 4H, $COC\boldsymbol{H}_2(CH_2)_4C\boldsymbol{H}_2CO$), 2.27 and 2.41 (m, 4H, 2 x $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 4H, 2 x >$NCOC\boldsymbol{H}_2$-cyclopentyl), 3.18 (m, 4H, 2 x $C^{\delta}\boldsymbol{H}_2$), 3.26 (m, 8H, 2 x $NH(C\boldsymbol{H}_2)_2NH$), 3.35-3.57 (m, 8H, 2 x 2/6-H piperazine), 3.46 and 3.51 (m, 4H, 2 x $NHC\boldsymbol{H}_2CH_2N$<), 3.77 (m, 4H, 2 x $NHCH_2C\boldsymbol{H}_2N$<), 4.22 (br, 2H, 2 x 11-H dibenzazepine-11-yl), 4.25 (m, 2H, 2 x $C^{\alpha}\boldsymbol{H}$), 7.12 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.20 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.28 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.32 (m, 10H, Ph), 7.37 (m, 10H, Ph), 7.41 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.49 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.79 (m, 2H, $\boldsymbol{H}_{Ar}$) ppm; MS (ES, +p) *m/z* (%): 1122 (10) $[M+2H]^{2+}$, 749 (75) $[M+3H]^{3+}$, 562 (100) $[M+4H]^{4+}$; RP-HPLC: 100 % (t_R = 20.2 min, *k´* = 6.5); $C_{120}H_{148}N_{26}O_{18}$ (2241.15).

***N*^{1},*N*^{30}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-amino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,11,20,26-tetroxo-6,10,21,25-tetraazatriacontanediamide (4.5).** ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.29 (m, 8H, $CO(CH_2)_2(CH_2)_4(CH_2)_2CO$), 1.51 and 1.64 (m, 8H, 2 x 3/4-H cyclopentyl), 1.51 and 1.67 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.76 (m, 4H, 2 x $C^{\beta}H_2$), 1.57 (m, 4H, $COCH_2CH_2(CH_2)_4CH_2CH_2CO$), 1.59 (m, 4H, 2 x $NHCH_2CH_2CH_2NH$), 1.60 (m, 4H, 2 x $C^{\gamma}H_2$), 1.92 (p, ^{3}J = 7.2 Hz, 4H, 2 x $COCH_2CH_2CH_2CO$), 2.16 (m, 4H, $COCH_2(CH_2)_6CH_2CO$), 2.11 and 2.17 (m, 8H, 2 x 3/5-H piperazine), 2.26 and 2.48 (m, 8H, 2 x $COCH_2CH_2CH_2CO$), 2.28 and 2.43 (m, 4H, 2 x $CH_2CON^{\alpha}H$), 2.49 (m, 4H, 2 x >$NCOCH_2$-cyclopentyl), 3.19 (m, 4H, 2 x $C^{\delta}H_2$), 3.20 (m, 8H, 2 x $NHCH_2CH_2CH_2NH$), 3.39 (br, 8H, 2 x 2/6-H piperazine), 3.45 and 3.55 (m, 4H, 2 x $NHCH_2CH_2N<$), 3.78 (m, 4H, 2 x $NHCH_2CH_2N<$), 4.25 (br, 4H, 2 x $C^{\alpha}H$, 2 x 11-H dibenzazepine-11-yl), 7.12 (m, 4H, H_{Ar}), 7.20 (m, 4H, H_{Ar}), 7.28 (m, 2H, H_{Ar}), 7.32 (m, 10H, Ph), 7.37 (m, 10H, Ph), 7.41 (m, 2H, H_{Ar}), 7.49 (m, 2H, H_{Ar}), 7.79 (m, 2H, H_{Ar}) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 21.5 (2 x $COCH_2CH_2CH_2CO$), 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.5 (2 x C^{γ}), 27.0 (2 x $NHCH_2CH_2CH_2NH$), 27.1 ($COCH_2CH_2(CH_2)_4CH_2CH_2CO$), 30.0 (2 x C^{β}), 30.3 ($CO(CH_2)_2(CH_2)_4(CH_2)_2CO$), 35.5 (2 x $COCH_2CH_2CH_2CO$), 36.9 and 37.2 ($COCH_2(CH_2)_6CH_2CO$), 37.8 (2 x $NHCH_2CH_2CH_2NH$), 38.4 (2 x $NHCH_2CH_2N<$), 39.4 and 39.5 (2 x C-2/5 cyclopentyl), 39.7 (2 x >$NCOCH_2$-cyclopentyl), 41.2 (2 x $NHCH_2CH_2N<$), 42.1 (2 x C^{δ}), 44.8 (2 x $CH_2CON^{\alpha}H$), 45.9 (2 x C-1 cyclopentyl), 47.6 (2 x C-2/6 piperazine), 52.2 and 52.6 (2 x C-3/5 piperazine), 53.7 (2 x C^{α}), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x $C_{Ar}H$), 124.1 (2 x Ph), 126.1 (2 x $C_{Ar}H$), 128.1 (2 x $C_{Ar}H$), 129.0 (2 x $C_{Ar}H$), 129.4 (2 x $C_{Ar}H$), 129.9 (2 x $C_{Ar}H$), 130.2 (2 x Ph), 131.1 (2 x $C_{Ar}H$), 131.7 ($C_{Ar}H$), 133.1 ($C_{Ar}H$), 137.8 (2 x C_{Ar}), 154.5 (2 x $N(CONPh)_2$), 154.8 (2 x $NC(N)N$), 172.7 (2 x $CON<$), 174.5 (2 x $CONH(CH_2)_2N<$), 174.7 (2 x $CON^{\alpha}H$), 175.0 (2 x $=NCO(CH_2)_3CO$), 176.3 (2 x $=NCO(CH_2)_3CO$), 176.4 ($CO(CH_2)_8CO$) ppm; a peak for C=O lactam could not be detected; MS (ES, +p) *m/z* (%): 1151 (25) $[M+2H]^{2+}$, 767 (95) $[M+3H]^{3+}$, 576 (100) $[M+4H]^{4+}$; RP-HPLC: 94 % (t_R = 14.3 min, k' = 4.3); $C_{124}H_{156}N_{26}O_{18}$ (2297.21).

***N*^{1},*N*^{30}-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-amino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,13,18,26-tetroxo-6,9,12,19,22,25-hexaazatriacontanediamide (4.6).** ^{1}H NMR (600 MHz, CD_3OD): δ = 1.49-1.80 (m, 28H, 2 x 3/4-H cyclopentyl, 2 x 2/5-H cyclopentyl, 2 x $C^{\beta}H_2C^{\gamma}H_2$, $COCH_2(CH_2)_2CH_2CO$), 1.93 (p, ^{3}J = 7.4 Hz, 4H, 2 x $COCH_2CH_2CH_2CO$), 2.00-2.20 (m, 8H, 2 x 3/5-H piperazine), 2.16 and 2.25 (2 x m, 4H, $COCH_2(CH_2)CH_2CO$), 2.27 and 2.41 (m, 4H, 2 x $CH_2CON^{\alpha}H$), 2.31 and 2.51 (t, ^{3}J = 7.4 Hz, 8H, 2 x $COCH_2CH_2CH_2CO$), 2.48 (m, 4H, 2 x >$NCOCH_2$-cyclopentyl), 3.18 (m, 4H, 2 x $C^{\delta}H_2$), 3.18 (m, 8H, 2 x $CONHCH_2CH_2NHCH_2$), 3.35-3.57 (m, 12H, 2 x 2/6-H piperazine, 2 x

NHC***H***$_2$CH$_2$N<), 3.47 (t, ^3J = 5.6 Hz, 8H, 4 x CONHC***H***$_2$), 3.77 (m, 4H, 2 x NHCH$_2$C***H***$_2$N<), 4.22 (br, 2H, 2 x 11-H dibenzazepine-11-yl), 4.25 (m, 2H, 2 x C$^{\alpha}$***H***), 7.12 (m, 4H, ***H***$_{Ar}$), 7.20 (m, 4H, ***H***$_{Ar}$), 7.28 (m, 2H, ***H***$_{Ar}$), 7.32 (m, 10H, Ph), 7.37 (m, 10H, Ph), 7.41 (m, 2H, ***H***$_{Ar}$), 7.49 (m, 2H, ***H***$_{Ar}$), 7.79 (m, 2H, ***H***$_{Ar}$) ppm; MS (ES, +p) *m/z* (%): 768 (30) [*M*+3H]$^{3+}$, 576 (100) [*M*+4H]$^{4+}$; RP-HPLC: 91 % (t_R = 16.9 min, *k´* = 5.3); $C_{122}H_{154}N_{28}O_{18}$ (2299.20).

N,N´*-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-6,11-dioxo-2,5,12,15-tetraazahexadecanediamide (4.7).** ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC, HMBC): δ = 1.49 and 1.62 (m, 8H, 2 x 3/4-H cyclopentyl), 1.49 and 1.65 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.73 (m, 4H, 2 x C$^{\beta}$H***$_2$), 1.57 (m, 4H, 2 x C$^{\gamma}$***H***$_2$), 1.59 (m, 4H, COCH$_2$(C***H***$_2$)$_2$CH$_2$CO), 2.10 and 2.15 (m, 8H, 2 x 3/5-H piperazine), 2.20 (m, 4H, COC***H***$_2$(CH$_2$)$_2$C***H***$_2$CO), 2.28 and 2.40 (m, 4H, 2 x C***H***$_2$CON$^{\alpha}$H), 2.46 (m, 4H, 2 x >NCOC***H***$_2$-cyclopentyl), 3.15 (m, 4H, 2 x C$^{\delta}$***H***$_2$), 3.28 (m, 4H, 2 x =NCONHC***H***$_2$CH$_2$NH), 3.30 (m, 4H, 2 x =NCONHCH$_2$C***H***$_2$NH), 3.38 (br, 8H, 2 x 2/6-H piperazine), 3.46 and 3.51 (m, 4H, 2 x NHC***H***$_2$CH$_2$N<), 3.77 (m, 4H, 2 x NHCH$_2$C***H***$_2$N<), 4.21 (s, 2H, 2 x 11-H dibenzazepine-11-yl), 4.25 (m, 2H, 2 x C$^{\alpha}$***H***), 7.11 (m, 4H, ***H***$_{Ar}$), 7.20 (m, 4H, ***H***$_{Ar}$), 7.27 (m, 2H, ***H***$_{Ar}$), 7.32 (m, 10H, Ph), 7.36 (m, 10H, Ph), 7.41 (m, 2H, ***H***$_{Ar}$), 7.49 (m, 2H, ***H***$_{Ar}$), 7.79 (m, 2H, ***H***$_{Ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.7 (2 x ***C***$^{\gamma}$), 26.2 (COCH$_2$(***C***H$_2$)$_2$CH$_2$CO), 30.0 (2 x ***C***$^{\beta}$), 36.7 (CO***C***H$_2$(CH$_2$)$_2$***C***H$_2$CO), 38.4 (2 x NH***C***H$_2$CH$_2$N<), 39.4 and 39.5 (2 x C-2/5 cyclopentyl), 39.7 (2 x >NCO***C***H$_2$-cyclopentyl), 39.8 (2 x =NCONHCH$_2$***C***H$_2$NH), 40.6 (2 x =NCONH***C***H$_2$CH$_2$NH), 41.2 (2 x NHCH$_2$***C***H$_2$N<), 41.9 (2 x ***C***$^{\delta}$), 44.8 (2 x ***C***H$_2$CON$^{\alpha}$H), 45.9 (2 x C-1 cyclopentyl), 47.6 (2 x C-2/6 piperazine), 52.2 and 52.6 (2 x C-3/5 piperazine), 53.7 (2 x ***C***$^{\alpha}$), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x ***C***$_{Ar}$H), 124.1 (2 x Ph), 126.0 (2 x ***C***$_{Ar}$H), 128.1 (2 x ***C***$_{Ar}$H), 128.9 (2 x ***C***$_{Ar}$H), 129.4 (2 x ***C***$_{Ar}$H), 129.9 (2 x ***C***$_{Ar}$H), 130.2 (2 x Ph), 131.0 (2 x ***C***$_{Ar}$H), 131.6 (***C***$_{Ar}$H), 132.5 (2 x ***C***$_{Ar}$), 133.1 (***C***$_{Ar}$H), 137.0 (2 x ***C***$_{Ar}$), 137.9 (2 x ***C***$_{Ar}$), 144.0 (2 x ***C***$_{Ar}$), 154.5 (2 x N(***C***ONPh)$_2$), 155.5 (2 x =N***C***ONH), 155.6 (2 x N***C***(N)N), 172.2 (2 x ***C***=O lactam), 172.6 (2 x ***C***ON<), 174.5 (2 x ***C***ONH(CH$_2$)$_2$N<), 174.7 (2 x ***C***ON$^{\alpha}$H), 176.3 (***C***O(CH$_2$)$_4$***C***O) ppm; MS (ES, +p) *m/z* (%): 1038 (30) [*M*+2H]$^{2+}$, 692 (100) [*M*+3H]$^{3+}$; RP-HPLC: 97 % (t_R = 14.6 min, *k´* = 4.4); $C_{110}H_{132}N_{26}O_{16}$ (2073.03).

N,N´*-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-6,13-dioxo-2,5,14,17-tetraazaoctadecanediamide (4.8).** ^{1}H NMR (600 MHz, CD$_3$OD, HSQC, HMBC): δ = 1.29 (m, 4H, CO(CH$_2$)$_2$(CH***$_2$)$_2$(CH$_2$)$_2$CO), 1.49 and 1.63 (m, 8H, 2 x 3/4-H cyclopentyl), 1.49 and 1.65 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.74 (m, 4H, 2 x C$^{\beta}$***H***$_2$), 1.57 (m, 4H, 2 x C$^{\gamma}$***H***$_2$), 1.57 (m, 4H, COCH$_2$C***H***$_2$(CH$_2$)$_2$C***H***$_2$CH$_2$CO), 2.11 and 2.16 (m, 8H, 2 x 3/5-H piperazine), 2.16 (m, 4H, COC***H***$_2$(CH$_2$)$_4$C***H***$_2$CO), 2.28 and 2.40 (m, 4H,

2 x C***H***$_2$CON$^{\alpha}$H), 2.46 (m, 4H, 2 x >NCOC***H***$_2$-cyclopentyl), 3.14 (m, 4H, 2 x C$^{\delta}$***H***$_2$), 3.28 (m, 4H, 2 x =NCONHC***H***$_2$CH$_2$NH), 3.29 (m, 4H, 2 x =NCONHCH$_2$C***H***$_2$NH), 3.38 (br, 8H, 2 x 2/6-H piperazine), 3.46 and 3.51 (m, 4H, 2 x NHC***H***$_2$CH$_2$N<), 3.77 (m, 4H, 2 x NHCH$_2$C***H***$_2$N<), 4.23 (s, 2H, 2 x 11-H dibenzazepine-11-yl), 4.25 (m, 2H, 2 x C$^{\alpha}$***H***), 7.12 (m, 4H, ***H***$_{Ar}$), 7.20 (m, 4H, ***H***$_{Ar}$), 7.30 (m, 2H, ***H***$_{Ar}$), 7.32 (m, 10H, Ph), 7.36 (m, 10H, Ph), 7.41 (m, 2H, ***H***$_{Ar}$), 7.49 (m, 2H, ***H***$_{Ar}$), 7.79 (m, 2H, ***H***$_{Ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.7 (2 x ***C***$^{\gamma}$), 26.7 (COCH$_2$***C***H$_2$(CH$_2$)$_2$***C***H$_2$CH$_2$CO), 29.9 (CO(CH$_2$)$_2$(***C***H$_2$)$_2$(CH$_2$)$_2$CO), 30.0 (2 x ***C***$^{\beta}$), 37.0 (CO***C***H$_2$(CH$_2$)$_4$***C***H$_2$CO), 38.5 (2 x NH***C***H$_2$CH$_2$N<), 39.4 and 39.5 (2 x C-2/5 cyclopentyl), 39.7 (2 x >NCO***C***H$_2$-cyclopentyl), 39.9 (2 x =NCONHCH$_2$***C***H$_2$NH), 40.6 (2 x =NCONH***C***H$_2$CH$_2$NH), 41.2 (2 x NHCH$_2$***C***H$_2$N<), 41.9 (2 x ***C***$^{\delta}$), 44.8 (2 x ***C***H$_2$CON$^{\alpha}$H), 46.0 (2 x C-1 cyclopentyl), 47.6 (2 x C-2/6 piperazine), 52.2 and 52.6 (2 x C-3/5 piperazine), 53.7 (2 x ***C***$^{\alpha}$), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x ***C***$_{Ar}$H), 124.1 (2 x Ph), 126.0 (2 x ***C***$_{Ar}$H), 128.1 (2 x ***C***$_{Ar}$H), 129.0 (2 x ***C***$_{Ar}$H), 129.4 (2 x ***C***$_{Ar}$H), 129.9 (2 x ***C***$_{Ar}$H), 130.2 (2 x Ph), 131.0 (2 x ***C***$_{Ar}$H), 131.6 (***C***$_{Ar}$H), 132.5 (2 x ***C***$_{Ar}$), 133.1 (***C***$_{Ar}$H), 136.9 (2 x ***C***$_{Ar}$), 137.9 (2 x ***C***$_{Ar}$), 154.5 (2 x N(***C***ONPh)$_2$), 155.5 (2 x =N***C***ONH), 155.6 (2 x N***C***(N)N), 172.1 (2 x ***C***=O lactam), 172.6 (2 x ***C***ON<), 174.5 (2 x ***C***ONH(CH$_2$)$_2$N<), 174.7 (2 x ***C***ON$^{\alpha}$H), 176.7 (***C***O(CH$_2$)$_6$***C***O) ppm; MS (ES, +p) *m/z* (%): 1053 (15) [*M*+2H]$^{2+}$, 702 (100) [*M*+3H]$^{3+}$; RP-HPLC: 98 % (t_R = 14.6 min, *k´* = 4.5); $C_{112}H_{136}N_{26}O_{16}$ (2101.06).

N*,*N´*-Bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-8,13-dioxo-2,7,14,19-tetraazaicosanediamide (4.9).** ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC, HMBC): δ = 1.49 and 1.62 (m, 8H, 2 x 3/4-H cyclopentyl), 1.50 and 1.66 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.73 (m, 4H, 2 x C$^{\beta}$H***$_2$), 1.50 (m, 8H, 2 x NHCH$_2$(C***H***$_2$)$_2$CH$_2$NH), 1.57 (m, 4H, 2 x C$^{\gamma}$***H***$_2$), 1.59 (m, 4H, COCH$_2$(C***H***$_2$)$_2$CH$_2$CO), 2.17 (m, 4H, COC***H***$_2$(CH$_2$)$_2$C***H***$_2$CO), 2.10 and 2.18 (m, 8H, 2 x 3/5-H piperazine), 2.26 and 2.41 (m, 4H, 2 x C***H***$_2$CON$^{\alpha}$H), 2.49 (m, 4H, 2 x >NCOC***H***$_2$-cyclopentyl), 3.13 (m, 4H, 2 x C$^{\delta}$***H***$_2$), 3.16 and 3.17 (m, 8H, 2 x NHC***H***$_2$(CH$_2$)$_2$C***H***$_2$NH), 3.31-3.47 (m, 8H, 2 x 2/6-H piperazine), 3.45 and 3.55 (m, 4H, 2 x NHC***H***$_2$CH$_2$N<), 3.78 (m, 4H, 2 x NHCH$_2$C***H***$_2$N<), 4.24 (br, 4H, 2 x C$^{\alpha}$***H***, 2 x 11-H dibenzazepine-11-yl), 7.12 (m, 4H, ***H***$_{Ar}$), 7.20 (m, 4H, ***H***$_{Ar}$), 7.27 (m, 2H, ***H***$_{Ar}$), 7.32 (m, 10H, Ph), 7.37 (m, 10H, Ph), 7.42 (m, 2H, ***H***$_{Ar}$), 7.50 (m, 2H, ***H***$_{Ar}$), 7.80 (m, 2H, ***H***$_{Ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.7 (2 x ***C***$^{\gamma}$), 26.6 (COCH$_2$(***C***H$_2$)$_2$CH$_2$CO), 27.9 (2 x NHCH$_2$(***C***H$_2$)$_2$CH$_2$NH), 30.0 (2 x ***C***$^{\beta}$), 36.8 (CO***C***H$_2$(CH$_2$)$_2$***C***H$_2$CO), 38.4 (2 x NH***C***H$_2$CH$_2$N<), 39.3 and 39.4 (2 x C-2/5 cyclopentyl), 39.7 (2 x >NCO***C***H$_2$-cyclopentyl), 39.9 and 40.4 (2 x NH***C***H$_2$(CH$_2$)$_2$***C***H$_2$NH), 41.2 (2 x NHCH$_2$***C***H$_2$N<), 41.8 (2 x ***C***$^{\delta}$), 44.7 (2 x ***C***H$_2$CON$^{\alpha}$H), 45.9 (2 x C-1 cyclopentyl), 47.5 (2 x C-2/6 piperazine), 52.2 and 52.6 (2 x C-3/5 piperazine), 53.7 (2 x ***C***$^{\alpha}$), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x ***C***$_{Ar}$H), 124.1 (2 x Ph), 126.1 (2 x ***C***$_{Ar}$H), 128.1 (2 x ***C***$_{Ar}$H), 129.0 (2 x ***C***$_{Ar}$H), 129.4 (2 x ***C***$_{Ar}$H), 129.9 (2 x

C$_{Ar}$H), 130.2 (2 x Ph), 131.1 (2 x ***C***$_{Ar}$H), 131.7 (***C***$_{Ar}$H), 132.5 (2 x ***C***$_{Ar}$), 133.1 (***C***$_{Ar}$H), 137.0 (2 x ***C***$_{Ar}$), 137.8 (2 x ***C***$_{Ar}$), 154.5 (2 x N(***C***ONPh)$_2$), 155.3 (2 x =N***C***ONH), 155.7 (2 x N***C***(N)N), 172.0 (2 x ***C***=O lactam), 172.7 (2 x ***C***ON<), 174.5 (2 x ***C***ONH(CH$_2$)$_2$N<), 174.7 (2 x ***C***ON$^{\alpha}$H), 175.8 and 179.3 (***C***O(CH$_2$)$_4$***C***O) ppm; MS (ES, +p) *m/z* (%): 1066 (25) [*M*+2H]$^{2+}$, 711 (100) [*M*+3H]$^{3+}$, 534 (90) [*M*+4H]$^{4+}$; RP-HPLC: 95 % (t_R = 20.8 min, *k´* = 6.7); $C_{114}H_{140}N_{26}O_{16}$ (2129.09).

18-(2-Aminoethyl)-*N*^{1},*N*^{35}-bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-5,10,14,22,26,31-hexaoxo-6,9,15,18,21,27,30-heptaazapentatriacontanediamide (4.10). ^{1}H NMR (600 MHz, CD$_3$OD, COSY, HSQC, HMBC): δ = 1.49 and 1.62 (m, 8H, 2 x 3/4-H cyclopentyl), 1.49 and 1.68 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.73 (m, 4H, 2 x C$^{\beta}$***H***$_2$), 1.60 (m, 4H, 2 x C$^{\gamma}$***H***$_2$), 1.87 and 1.91 (m, 8H, 4 x COCH$_2$C***H***$_2$CH$_2$CO), 2.11 and 2.18 (m, 8H, 2 x 3/5-H piperazine), 2.21 and 2.25 and 2.41 and 2.48 (m, 16H, 4 x COC***H***$_2$CH$_2$C***H***$_2$CO), 2.27 and 2.41 (m, 4H, 2 x C***H***$_2$CON$^{\alpha}$H), 2.48 (m, 4H, 2 x >NCOC***H***$_2$-cyclopentyl), 2.74 (br, 4H, NHCH$_2$C***H***$_2$N(C$_2$H$_4$NH$_2$)C***H***$_2$CH$_2$NH), 3.06 (m, 4H, >N(C***H***$_2$)$_2$NH$_2$), 3.26 and 3.83 (m, 8H, 2 x NH(C***H***$_2$)$_2$NH), 3.18 (m, 4H, 2 x C$^{\delta}$***H***$_2$), 3.28 (m, 4H, NHC***H***$_2$CH$_2$N(C$_2$H$_4$NH$_2$)CH$_2$C***H***$_2$NH), 3.31-3.48 (m, 8H, 2 x 2/6-H piperazine), 3.45 and 3.53 (m, 4H, 2 x NHC***H***$_2$CH$_2$N<), 3.78 (m, 4H, 2 x NHCH$_2$C***H***$_2$N<), 4.25 (m, 4H, 2 x C$^{\alpha}$***H***, 2 x 11-H dibenzazepine-11-yl), 7.12 (m, 4H, ***H***$_{Ar}$), 7.20 (m, 4H, ***H***$_{Ar}$), 7.27 (m, 2H, ***H***$_{Ar}$), 7.32 (m, 10H, Ph), 7.37 (m, 10H, Ph), 7.42 (m, 2H, ***H***$_{Ar}$), 7.50 (m, 2H, ***H***$_{Ar}$), 7.80 (m, 2H, ***H***$_{Ar}$) ppm; ^{13}C NMR (150 MHz, CD$_3$OD, HSQC, HMBC): δ = 21.4 and 23.0 (4 x COCH$_2$***C***H$_2$CH$_2$CO), 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.4 (2 x ***C***$^{\gamma}$), 30.0 (2 x ***C***$^{\beta}$), 35.6 and 36.2 and 36.8 (4 x CO***C***H$_2$CH$_2$***C***H$_2$CO), 38.1 (>NCH$_2$***C***H$_2$NH$_2$), 38.3 (2 x NH(***C***H$_2$)$_2$NH), 38.4 (2 x NH***C***H$_2$CH$_2$N<), 39.2 and 39.3 (2 x C-2/5 cyclopentyl), 39.5 (2 x >NCO***C***H$_2$-cyclopentyl), 39.8 (NH***C***H$_2$CH$_2$N(C$_2$H$_4$NH$_2$)CH$_2$***C***H$_2$NH), 41.2 (2 x NHCH$_2$***C***H$_2$N<), 42.0 (2 x ***C***$^{\delta}$), 44.7 (2 x ***C***H$_2$CON$^{\alpha}$H), 45.9 (2 x C-1 cyclopentyl), 47.5 (2 x C-2/6 piperazine), 52.1 and 52.6 (2 x C-3/5 piperazine), 53.7 (2 x ***C***$^{\alpha}$), 55.6 (NHCH$_2$***C***H$_2$N(C$_2$H$_4$NH$_2$)***C***H$_2$CH$_2$NH), 59.5 (>N***C***H$_2$CH$_2$NH$_2$), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x ***C***$_{Ar}$H), 124.1 (2 x Ph), 126.0 (2 x ***C***$_{Ar}$H), 128.1 (2 x ***C***$_{Ar}$H), 129.0 (2 x ***C***$_{Ar}$H), 129.4 (2 x ***C***$_{Ar}$H), 129.9 (2 x ***C***$_{Ar}$H), 130.2 (2 x Ph), 131.1 (2 x ***C***$_{Ar}$H), 131.7 (***C***$_{Ar}$H), 132.3 (2 x ***C***$_{Ar}$), 133.2 (***C***$_{Ar}$H), 136.8 (2 x ***C***$_{Ar}$), 137.8 (2 x ***C***$_{Ar}$), 154.5 (2 x N(***C***ONPh)$_2$), 155.0 (2 x N***C***(N)N), 172.7 (2 x ***C***ON<), 174.5 (2 x ***C***ONH(CH$_2$)$_2$N<), 174.7 (2 x ***C***ON$^{\alpha}$H), 175.4 and 175.8 and 176.4 (2 x =N***C***O(CH$_2$)$_3$***C***ONH, 2 x NH***C***O(CH$_2$)$_3$***C***ONH) ppm; a peak for C=O lactam could not be detected; MS (ES, +p) *m/z* (%): 815 (35) [*M*+3H]$^{3+}$, 612 (100) [*M*+4H]$^{4+}$, 490 (40) [*M*+5H]$^{5+}$; RP-HPLC: 98 % (t_R = 16.9 min, *k´* = 5.2); $C_{128}H_{164}N_{30}O_{20}$ (2441.27).

16-(2-Aminoethyl)-*N*,*N*′-bis(amino{(4*S*)-5-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethylamino]-5-oxo-4-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetamido]pentylamino}methylene)-8,12,20,24-tetroxo-2,7,13,16,19,25,30-heptaazahentriacontanediamide (4.11). ^{1}H NMR (600 MHz, CD_3OD, COSY, HSQC, HMBC): δ = 1.50 (m, 8H, 2 x $NHCH_2(C\boldsymbol{H}_2)_2CH_2NH$), 1.50 and 1.62 (m, 8H, 2 x 3/4-H cyclopentyl), 1.50 and 1.68 (m, 8H, 2 x 2/5-H cyclopentyl), 1.51 and 1.75 (m, 4H, 2 x $C^{\beta}\boldsymbol{H}_2$), 1.57 (m, 4H, 2 x $C^{\gamma}\boldsymbol{H}_2$), 1.87 (m, 4H, 2 x $COCH_2C\boldsymbol{H}_2CH_2CO$), 2.13 and 2.19 (m, 8H, 2 x 3/5-H piperazine), 2.19 and 2.23 (m, 8H, 2 x $COC\boldsymbol{H}_2CH_2C\boldsymbol{H}_2CO$), 2.27 and 2.41 (m, 4H, 2 x $C\boldsymbol{H}_2CON^{\alpha}H$), 2.48 (m, 4H, 2 x >$NCOC\boldsymbol{H}_2$-cyclopentyl), 2.79 (br, 4H, $NHCH_2C\boldsymbol{H}_2N(C_2H_4NH_2)C\boldsymbol{H}_2CH_2NH$), 3.14 (m, 4H, 2 x $C^{\delta}\boldsymbol{H}_2$), 3.16 (m, 4H, >$N(C\boldsymbol{H}_2)_2NH_2$), 3.17 (m, 8H, 2 x $NHC\boldsymbol{H}_2(CH_2)_2C\boldsymbol{H}_2NH$), 3.31 (m, 4H, $NHC\boldsymbol{H}_2CH_2N(C_2H_4NH_2)CH_2C\boldsymbol{H}_2NH$), 3.32-3.48 (m, 8H, 2 x 2/6-H piperazine), 3.46 and 3.53 (m, 4H, 2 x $NHC\boldsymbol{H}_2CH_2N$<), 3.77 (m, 4H, 2 x $NHCH_2C\boldsymbol{H}_2N$<), 4.26 (m, 4H, 2 x $C^{\alpha}\boldsymbol{H}$, 2 x 11-H dibenzazepine-11-yl), 7.13 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.20 (m, 4H, $\boldsymbol{H}_{Ar}$), 7.29 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.32 (m, 10H, Ph), 7.36 (m, 10H, Ph), 7.42 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.50 (m, 2H, $\boldsymbol{H}_{Ar}$), 7.80 (m, 2H, $\boldsymbol{H}_{Ar}$) ppm; ^{13}C NMR (150 MHz, CD_3OD, HSQC, HMBC): δ = 23.2 (2 x $COCH_2\boldsymbol{C}H_2CH_2CO$), 24.5 and 24.6 (2 x C-3/4 cyclopentyl), 25.7 (2 x $\boldsymbol{C}^{\gamma}$), 27.7 and 27.9 (2 x $NHCH_2(\boldsymbol{C}H_2)_2CH_2NH$), 29.9 (2 x $\boldsymbol{C}^{\beta}$), 36.2 and 36.3 (2 x $CO\boldsymbol{C}H_2CH_2\boldsymbol{C}H_2CO$), 38.2 ($NH\boldsymbol{C}H_2CH_2N(C_2H_4NH_2)CH_2\boldsymbol{C}H_2NH$), 38.4 (2 x $NH\boldsymbol{C}H_2CH_2N$<), 39.2 and 39.3 (2 x C-2/5 cyclopentyl), 39.5 (2 x >$NCO\boldsymbol{C}H_2$-cyclopentyl), 39.9 (>$NCH_2\boldsymbol{C}H_2NH_2$), 40.4 (2 x $NH\boldsymbol{C}H_2(CH_2)_2\boldsymbol{C}H_2NH$), 41.2 (2 x $NHCH_2\boldsymbol{C}H_2N$<), 41.8 (2 x $\boldsymbol{C}^{\delta}$), 44.7 (2 x $\boldsymbol{C}H_2CON^{\alpha}H$), 45.9 (2 x C-1 cyclopentyl), 47.4 (2 x C-2/6 piperazine), 52.2 and 52.6 (2 x C-3/5 piperazine), 53.6 (2 x $\boldsymbol{C}^{\alpha}$), 55.8 ($NHCH_2\boldsymbol{C}H_2N(C_2H_4NH_2)\boldsymbol{C}H_2CH_2NH$), 59.5 (>$N\boldsymbol{C}H_2CH_2NH_2$), 75.8 (2 x C-11 dibenzazepine-11-yl), 123.0 (2 x $\boldsymbol{C}_{Ar}H$), 124.1 (2 x Ph), 126.0 (2 x $\boldsymbol{C}_{Ar}H$), 128.1 (2 x $\boldsymbol{C}_{Ar}H$), 129.0 (2 x $\boldsymbol{C}_{Ar}H$), 129.4 (2 x $\boldsymbol{C}_{Ar}H$), 129.9 (2 x $\boldsymbol{C}_{Ar}H$), 130.2 (2 x Ph), 131.1 (2 x $\boldsymbol{C}_{Ar}H$), 131.7 ($\boldsymbol{C}_{Ar}H$), 132.4 (2 x $\boldsymbol{C}_{Ar}$), 133.2 ($\boldsymbol{C}_{Ar}H$), 136.9 (2 x $\boldsymbol{C}_{Ar}$), 137.9 (2 x $\boldsymbol{C}_{Ar}$), 154.5 (2 x $N(\boldsymbol{C}ONPh)_2$), 155.4 (2 x =$N\boldsymbol{C}ONH$), 155.7 (2 x $N\boldsymbol{C}(N)N$), 172.7 (2 x $\boldsymbol{C}ON$<), 174.5 (2 x $\boldsymbol{C}ONH(CH_2)_2N$<), 174.7 (2 x $\boldsymbol{C}ON^{\alpha}H$), 175.2 and 176.2 (2 x $NH\boldsymbol{C}O(CH_2)_3\boldsymbol{C}ONH$) ppm; a peak for C=O lactam could not be detected; MS (ES, +p) *m/z* (%): 787 (50) $[M+3H]^{3+}$, 591 (100) $[M+4H]^{4+}$, 473 (40) $[M+5H]^{5+}$; RP-HPLC: 95 % (t_R = 17.3 min, k' = 5.4);$C_{124}H_{160}N_{30}O_{18}$ (2357.25).

4.5.3 Pharmacology: Cell Culture, Fura-2 Assay and Flow Cytometry

Cell culture. CHO cells were cultured as described elsewhere.[45]

Fura-2 assay on CHO cells. The fura-2 assay was performed as previously described[46] using a LS50 B spectrofluorimeter from Perkin Elmer (Überlingen, Germany).

Flow cytometric competition binding assay. The binding assay was performed as described elsewhere[45] with the adaptations according to Chapter 3.5.4.

Flow cytometric selectivity assay. The selectivity was determined as described in Chapter 3.5.4.

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Chapter 5 Red-Fluorescent NPY Y_2 Receptor Antagonists

5.1 Introduction

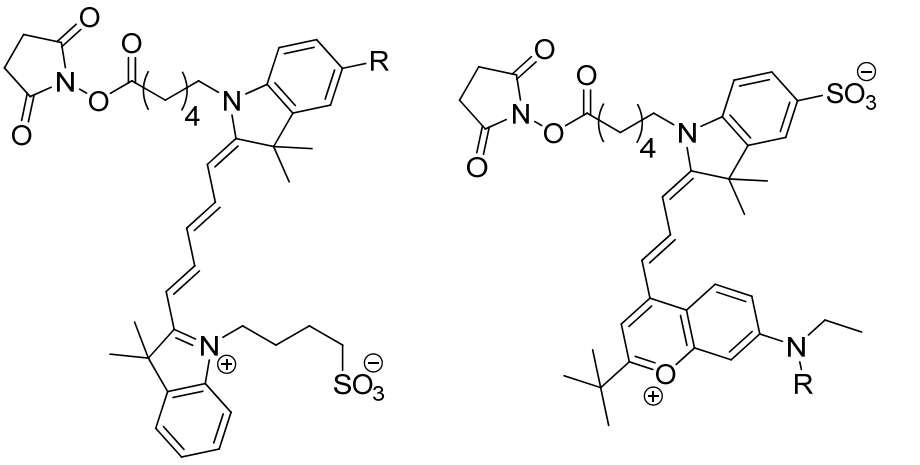
There has been a remarkable growth in the use of fluorescence-based techniques for the investigation of ligand-receptor interactions over the past two decades. In the field of G-protein coupled receptor (GPCR) research, recent advances in both labeling methods and measuring techniques have made fluorescence assays indispensable tools for the exploration and visualization of GPCRs.[1-2] The receptors can be labeled, for instance, with auto-fluorescent proteins, e. g., for direct detection *in vivo*, as well as for the investigation of protein-protein-interactions by the measurement of fluorescence-resonance energy transfer (FRET) of an appropriate donor-acceptor pair.[3-5] Moreover, indirect labeling with fluorescence ligands is commonly used to explore ligand-receptor interactions and to visualize GPCRs without modification of the receptor protein.

In the field of peptidergic GPCRs, fluorescence-labeled endogenous ligands are often used. In the case of peptides the decrease in affinity caused by attachment of a fluorophore is usually rather low, as the structures of such larger molecules are only moderately changed and the interactions with the receptor are hardly affected by the introduction of the dye. There are several examples in the literature for the application of NPY fluorescent probes.[6-8] Such fluorescent NPY analogs can be utilized, e.g. for internalization studies by means of confocal microscopy or flow cytometry[9] and as standard ligand in fluorescence-based binding experiments[10], respectively. The synthesis of fluorescent small-molecule ligands for GPCRs is by far more challenging. Herein, a potential site for fluorophore conjugation is in much closer proximity to the pharmacophore and as a consequence, much more likely to affect ligand affinity and efficacy. Nevertheless, there are several examples for the successful development of low molecular weight fluorescent ligands and their application in the investigation of GPCRs.[11-19]

Usually, endogenous ligands and their labeled analogues do not discriminate between subtypes of the GPCRs of interest. For the detection and investigation of receptor subtypes on cells and in tissues selective high affinity fluorescence ligands are required. Fluorescent non-peptide antagonists are superior to peptides concerning selectivity, kinetics, stability and mode of action. Antagonists do not induce receptor internalization, which may complicate the determination of binding kinetics and increase non-specific binding due to intracellular accumulation, thus,

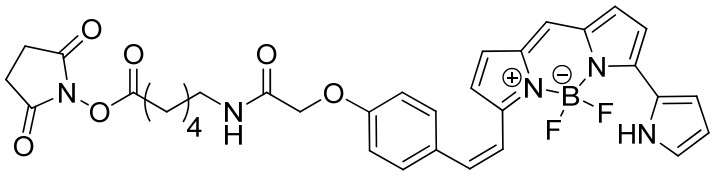
leading to a high fluorescence background, e.g., in flow cytometric binding assays. Additionally, peptides show slower kinetics and hence longer incubation periods are needed. Besides, peptides are susceptible to enzymatic cleavage.

The lack of selective non-peptide fluorescent ligands for the Y_2R prompted us to develop fluorescent probes starting from BIIE 0246,[20] the first highly potent selective Y_2R antagonist. The guanidine-acylguanidine bioisosteric approach, which was successfully applied in our group for the synthesis of fluorescent Y_1R antagonists,[12] is an efficient strategy to combine labeling with lowering of the basicity of guanidine containing GPCR ligands. Therefore, the applicability of this concept to argininamide-type Y_2R antagonists was explored aiming at the development of potent and selective fluorescent ligands.

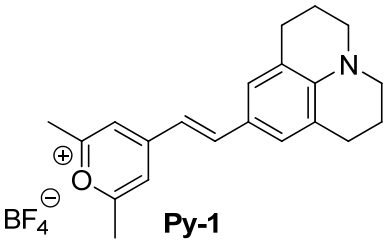


S0436: R = H
S0387: R = SO_3^-

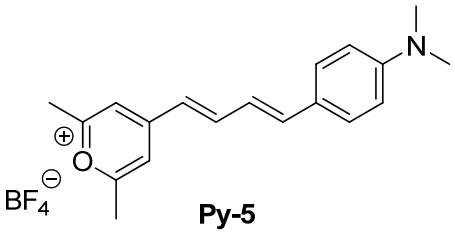
Dy-630: R = Et
Dy-633: R = $(CH_2)_3SO_3^-$



Bodipy 650/665-X ("Bodipy")



Py-1



Py-5

Figure 5.1. Structures of the dyes used for the preparation of the red-fluorescent Y_2R antagonists (**5.1**-**5.27**).

5.2 Chemistry

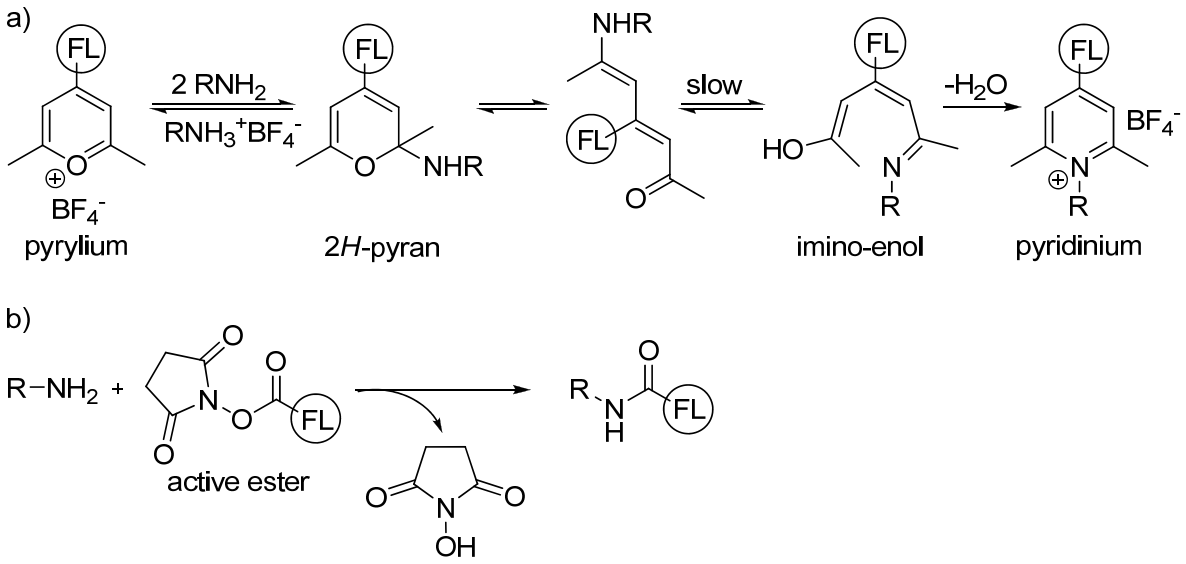
5.2.1 Synthesis

With respect to potential use in cellular assays red fluorescent dyes depicted in Figure 5.1 with a fluorescence emission wavelength over 590 nm were selected to reduce the background fluorescence and thus to improve the signal-to-noise ratio. Preferably, we used the new class of pyrylium dyes (Py-1 and Py-5)[21-22] as well as the Cy5[23]-related cyanine dyes S0436[19] and S0387. Furthermore, the Dyomics dyes Dy-630[24] and Dy-633[25-26] with an unsymmetrical hemicyanine structure consisting of an indol and a benzopyrane moiety, and the boron-containing Bodipy® 650/665-X dye[27] were applied in order to expand the structural diversity of fluorescent Y_2R antagonists.

The (hemi-)cyanine dyes and the Bodipy dye, respectively, are excitable at 635 nm with a red diode laser,[28] commonly used as light source in commercial flow cytometers. The pyrylium dyes (Py-1 and Py-5) belong to the new class of "chameleon labels", which has been developed for the staining and quantification of proteins at picomolar concentrations.[21] These dyes widen the pharmacological applicability of fluorescent ligands, as they are excitable at lower wavelengths by a widely used argon laser (λ = 488 nm). Thus, Py-labeled compounds and, e.g., cyanine fluorescent ligands can be applied simultaneously in cellular assays. Another advantage of pyrylium dyes is their lower molecular weight compared to other dye classes. In this case especially coupling to small molecules should be more tolerated, as the affinity-lowering influence of the fluorophore should be less pronounced.

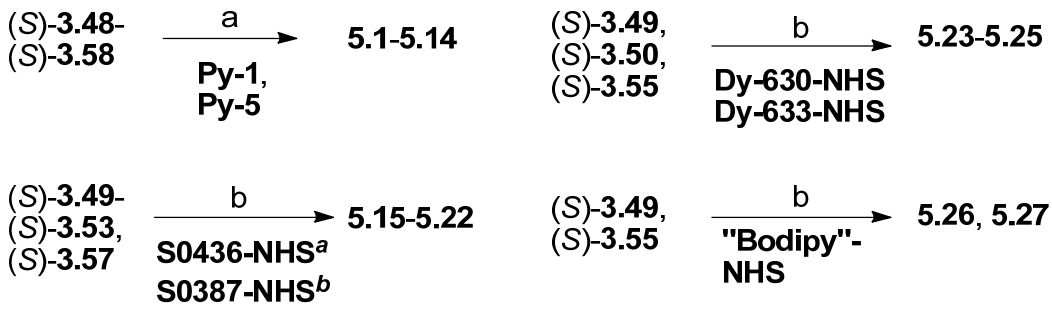
The pyrylium dyes react very rapidly and regioselectively with primary amines at pH 8-9 at room temperature to form the resonance-stabilized pyridinium compound with retained charge at the nitrogen. This ring transformation is accompanied by a hypsochromic shift of the absorption maximum by more than 100 nm (621 nm to 503 nm for Py-1; 644 nm to 465 nm for Py-5).[22] Thus, the reaction can be followed visually due to a change in color from blue to red. The mechanism of the pyrylium-pyridinium conversion has been studied extensively (Scheme 5.1a).[29] Addition of the primary amine at position 2 yields the 2*H*-pyran. This intermediate is energetically unfavored in terms of aromaticity and hence the ring opens to form the second intermediate, a divinylogous amide which is usually more stable. This amide tautomerizes in the rate-determining step into an imino-enol which rapidly cyclizes into the pyridinium product.

The other dyes (S0436, S0387, Dy-630, Dy-633, Bodipy® 650/665) contain a hexanoyl side chain, which enables coupling to amines *via* the succinimidyl active esters (Scheme 5.1b).



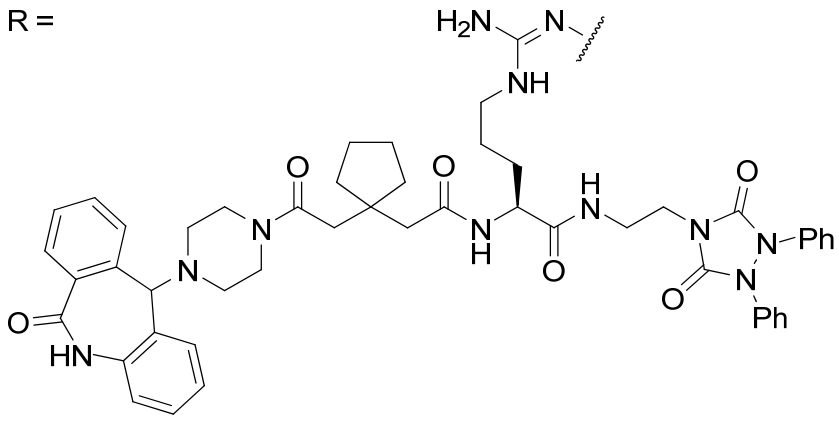
Scheme 5.1. a) General mechanism for the reaction of pyrylium dyes with primary amines. b) Principle of the acylation of primary amines with fluorescent active esters; (FL: Fluorophore).

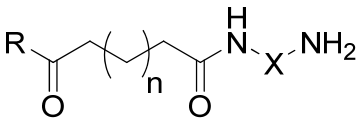
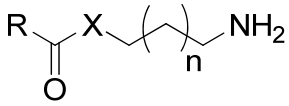
For the preparation of fluorescent Y_2R antagonists derived from BIIE 0246 the unprotected amine precursors (*S*)-**3.48**-(*S*)-**3.58** (for the preparation cf. chapter 3) were used. Labeling with the aforementioned amino-reactive dyes under basic conditions yielded compounds **5.1**-**5.27** (Scheme 5.2). Coupling of Py-5 and S0436-NHS, respectively, to the amine precursor (*S*)-**3.60** resulted in fluorescent ligands **5.28** and **5.29** with reduced lipophilicity in the ornithinamide building block as well as an additional positive charge in the acyllinker (Scheme 5.3). The primary amine in the acyl- or carbamoyllinker is more reactive and sterically well accessible compared with the free guanidino group. Therefore, the products were obtained in satisfactory yields despite the lack of protection at the free $N^{\omega'}H_2$. The labeling reactions were stopped with 10 % aq. TFA and the crude products were purified by preparative HPLC.

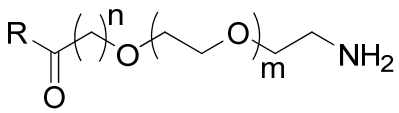


For structures of the fluorescent ligands **5.1**-**5.27** cf. Table 5.3

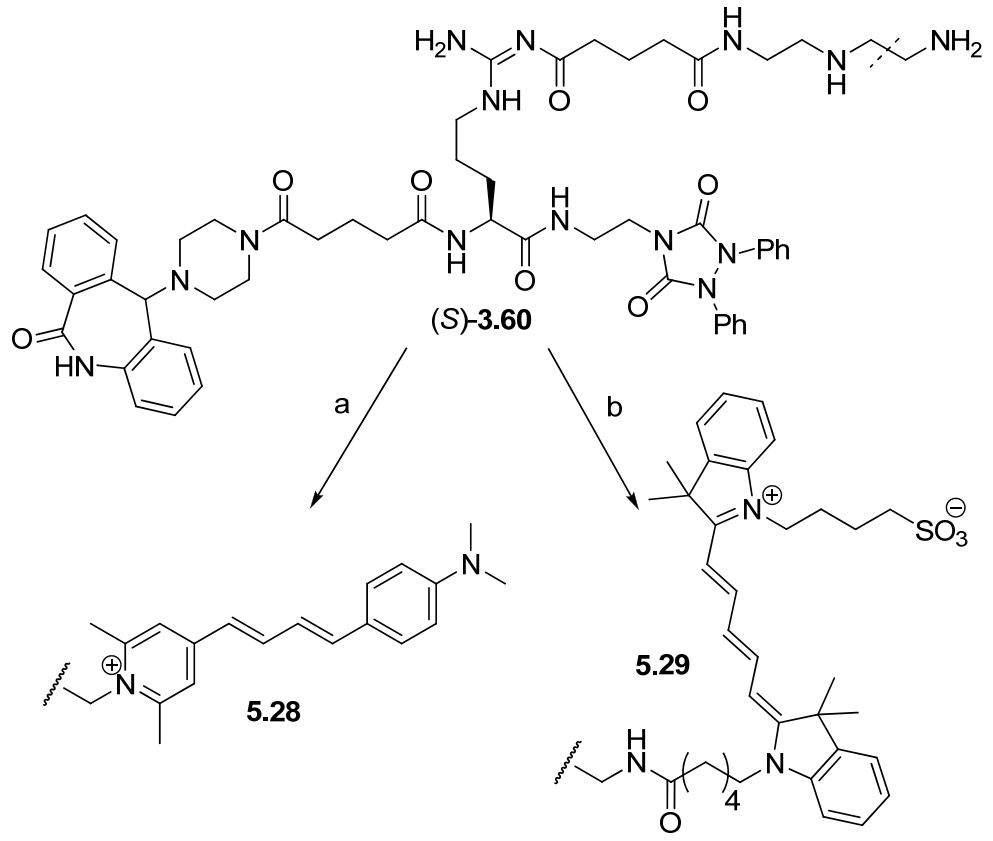
R =



	No	X	n
	(*S*)-**3.48**	$(CH_2)_2$	1
	(*S*)-**3.49**	$(CH_2)_3$	1
	(*S*)-**3.50**	$(CH_2)_2NH(CH_2)_2$	1
	(*S*)-**3.51**	$CH_2(CH_2CH_2O)_3(CH_2)_3$	0
	(*S*)-**3.52**	NH	0
	(*S*)-**3.53**	NH	1
	(*S*)-**3.54**	NH	2
	(*S*)-**3.55**	NH	4
	(*S*)-**3.56**	CH_2	2

	No	n	m
	(*S*)-**3.57**	1	1
	(*S*)-**3.58**	2	3

Scheme 5.2. Preparation of the fluorescent Y_2R antagonists **5.1**-**5.27** derived from BIIE 0246. Reagents and conditions: a) Pyrylium dye (1 eq), amine precursor (4 eq), NEt_3 (15 eq), CH_3CN, DMF, 1-2 h, rt; b) pertinent dye succinimidyl ester (1 eq), amine precursor (2 eq), NEt_3 (10-15 eq), CH_3CN, DMF, 7-16 h, rt. [a] The succinimidyl ester of S0436 was purchased from FEW Chemicals (Germany) under the name S0536. [b] The succinimidyl ester of S0387 was purchased from FEW Chemicals (Germany) under the name S0586.



Scheme 5.3. Synthesis of the fluorescence ligands **5.28** and **5.29**. Reagents and conditions: a) Py-5 (1 eq), (*S*)-**3.60** (3 eq), NEt_3 (15 eq), CH_3CN, DMF, 1 h, rt; b) S0436-NHS (1 eq), (*S*)-**3.60** (3 eq), NEt_3 (10 eq), CH_3CN, DMF, 16 h, rt.

5.2.2 Fluorescence Properties of the Labeled Y_2R Antagonists

The absorption and emission spectra of the fluorescent ligands were measured in three different solvents: in phosphate buffered saline (PBS) at pH 7.0, in PBS with 1 % of bovine serum albumin (BSA) and in ethanol (EtOH). The fluorescence quantum yields (QY; φ) were determined with cresyl violet perchlorate as reference. PBS represents a commonly used standard solvent. By the addition of 1 % of BSA, assay conditions are simulated and the influence of proteins on the spectral properties are taken into account, whereas the determination in EtOH is regarded useful to observe the influence of the polarity of the solvent (solvatochromism). The fluorescence properties of the labeled Y_2R antagonists are summarized in Table 5.1. Generally, the chemical structure of the linkers, connecting the fluorophore with the pharmacophoric moiety, has almost no effect on the fluorescence properties.

The majority of fluorescent ligands shows very high QYs (up to circa 50 %) in PBS + 1 % BSA and very low QYs in pure PBS (< 6.2 %) except for compounds **5.17** and **5.23** (11-13 % in PBS). The increase in QY by a factor of around 10 for cyanine dyes and by up to over 100 in case of pyrylium coupled fluorophores after the addition of BSA results from intermolecular interactions of the fluorophores with the protein. Binding of the fluorescent ligands to proteins leads to a rigidization of the molecule, which is

followed by an increase in fluorescence intensity and therefore in QY.[30] Thus, when BSA-free buffers are used for flow cytometric binding assays, the fluorescence intensity of these fluorescent ligands would probably increase in the receptor bound state. Nevertheless, an increase in fluorescence intensity could also be related to non-specific interactions with other proteins or with the cell membrane. Furthermore, the protein binding to BSA is accompanied by a hypsochromic shift of the emission maxima of Py-labeled ligands, presumably, due to reduced molecular vibrations. The Bodipy derivatives **5.26** and **5.27** have exceptionally poor quantum yields in PBS + 1 % BSA. Presumably, the spectroscopic properties of the Bodipy dyes are less affected by protein binding and rigidization.

Table 5.1. Spectroscopic properties of the fluorescent Y_2R antagonists **5.1**-**5.18**, **5.20**-**5.27**: Influence of the polarity of the solvent (PBS, pH = 7.0 vs. EtOH) and protein (BSA) on the quantum yield φ (reference: cresyl violet perchlorate) and on the excitation and emission maxima (λ_{ex}, λ_{em}), respectively.

No	Dye	PBS		PBS + 1 % BSA		EtOH	
		λ_{ex} / λ_{em} / nm	φ / %	λ_{ex} / λ_{em} / nm	φ / %	λ_{ex} / λ_{em} / nm	φ / %
5.1	Py-1	511 / 636	0.6	513 / 609	50	523 / 634	2.3
5.2	Py-1	522 / 642	0.3	511 / 606	43	523 / 632	1.8
5.3	Py-5	490 / 706	2.2	487 / 634	35	505 / 703	19
5.4	Py-1	525 / 643	0.9	514 / 612	39	527 / 636	1.9
5.5	Py-1	505 / 638	1.4	511 / 608	37	519 / 631	2.0
5.6	Py-1	532 / 641	0.5	536 / 619	43	--	--
5.7	Py-1	516 / 640	0.5	519 / 608	49	525 / 634	1.9
5.8	Py-5	489 / 702	0.5	492 / 640	27	505 / 705	18
5.9	Py-1	515 / 641	1.2	515 / 609	47	524 / 634	2.4
5.10	Py-5	490 / 704	1.4	485 / 641	36	502 / 705	23
5.11	Py-1	515 / 645	0.6	516 / 608	56	522 / 634	2.1
5.12	Py-5	485 / 705	1.1	488 / 642	44	502 / 706	24
5.13	Py-1	516 / 641	0.6	517 / 612	44	525 / 635	2.2
5.14	Py-5	475 / 707	5.1	490 / 644	32	502 / 705	22
5.15	S0436	661 / 664	1.6	660 / 677	40	651 / 673	34
5.16	S0387	657 / 667	4.9	660 / 677	34	654 / 675	37
5.17	S0436	649 / 663	13	659 / 676	36	651 / 674	30
5.18	S0387	654 / 667	6.2	658 / 675	47	654 / 676	35
5.20	S0436	663 / 664	2.5	659 / 674	32	653 / 672	44
5.21	S0387	654 / 667	3.3	659 / 675	36	654 / 675	41
5.22	S0436	658 / 664	1.4	661 / 675	28	652 / 674	30
5.23	Dy-633	634 / 652	11	644 / 656	55	640 / 660	24
5.24	Dy-630	651 / 655	1.7	644 / 662	52	640 / 662	11
5.25	Dy-633	653 / 655	0.5	647 / 659	23	639 / 660	21
5.26	“Bodipy”	671 / --	(0.05)	667 / 670	5.6	--	--
5.27	“Bodipy”	673 / --	(0.1)	665 / 671	18	652 / 668	34

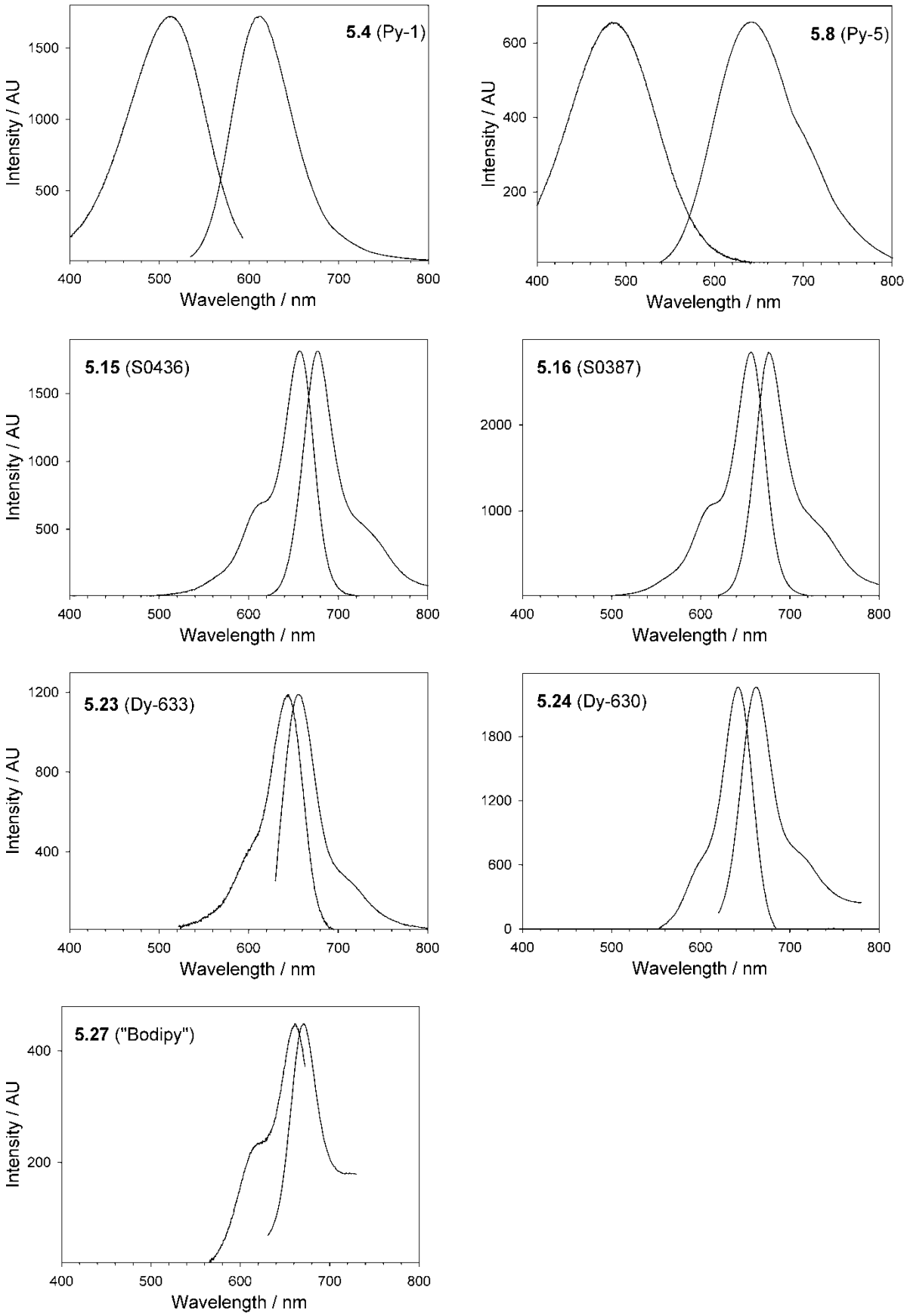


Figure 5.2. Absorption and corrected emission spectra in PBS + 1 % BSA exemplified for Py-1, Py-5, S0436, S0387, Dy-630, Dy-633 and Bodipy 650/665 ("Bodipy") labeled antagonists (recorded at 22 °C).

In EtOH, the differences in QYs of the various fluorophores are much more pronounced. Whereas the Py-1 labeled antagonists hold very low QYs in the same range as in PBS, the Py-5 as well as the Dy-630 and Dy-633 containing ligands, respectively, exhibit moderate QYs (up to 24 % for **5.12** and **5.23**). The cyanine labeled ligands **5.15**-**5.22** display QYs up to 44 %, which is comparable to the yields measured in PBS + 1 % BSA. Lastly, the Bodipy-labeled analog **5.27** even shows a higher QY in EtOH, than in PBS containing BSA. Obviously, Py-1 labeled Y_2R antagonists are less sensitive towards hydrophobic interactions compared to the ligands labeled with other dyes.

The absorption and corrected emission spectra of various fluorescence labeled Y_2R antagonists in PBS containing 1 % of BSA are depicted in Figure 5.2. The Py-5 labeled ligands show the largest Stokes shift (≈ 150 nm; in pure PBS more than 200 nm) followed by the Py-1 derivatives (≈ 100 nm). Hence, the pyrylium coupled red-fluorescent ligands can be excited by the argon laser at 488 nm. The cyanine- (S0436, S0387), hemicyanine- (Dy-630, Dy-633), and Bodipy-labeled antagonists reveal a much smaller Stokes shift and are excitable at 635 nm with the red diode laser, commonly used in flow cytometry.

In conclusion, the fluorescence properties of all synthesized fluorescent ligands allow for an application to flow cytometric equilibrium binding studies and confocal microscopy. It is noticeable that the pyridinium fluorophores can be investigated in the presence of, e.g., cyanine-labeled fluorescent probes and *vice versa* due to different excitation wavelengths.

5.2.3 Stability of the Fluorescence Ligands

As already discussed in Chapter 3, N^{ω}-acylated argininamides were recently reported to be problematic in terms of stability of the acylguanidine side chain.[31-32] However, HPLC analysis revealed high stability for the majority of the investigated Y_2R antagonists under assays-like conditions over a period of (at least) 90 minutes (Chapter 3.2.2). Especially, the acylation of the amine precursor appeared to be beneficial in terms of stability. Therefore, compounds labeled with pyrylium dyes were of special interest in this investigation due to their preserved positive charge in the linker. The percentage of decomposition resulting in BIIE 0246 was determined at physiological pH (Table 5.2).

The Py-5 labeled antagonists **5.10** and **5.14** comprising a positive charge in the fluorophore moiety proved to be stable. Even compound **5.4** including a secondary amine in the linker showed a very low decomposition giving BIIE 0246 (1.5 %; Figure 5.3). Interestingly, the amount of formed BIIE 0246 was even lower with respect to the appropriate amine precursor (*S*)-**3.50** (2.9 %), comprising two reactive amino moieties in the linker. Moreover, the S0436 labeled fluorescent ligands **5.17** and **5.22** exhibited a high stability within 90 minutes. In conclusion, the affinities examined in

the flow cytometric equilibrium binding assay are supposed to be not affected by the formation of BIIE 0246.

Table 5.2. Chemical stability (aqueous buffer, pH 7.4, 20 °C, 90 min) of the fluorescent Y_2R antagonists.

No.	% Decomposition	No.	% Decomposition
5.4	1.5	**5.17**	1.0
5.10	1.8	**5.22**	1.5
5.14	1.0		

Cleavage of the acyl or carbamoyl substituent from the guanidine moiety resulting in BIIE 0246 was analyzed by HPLC.

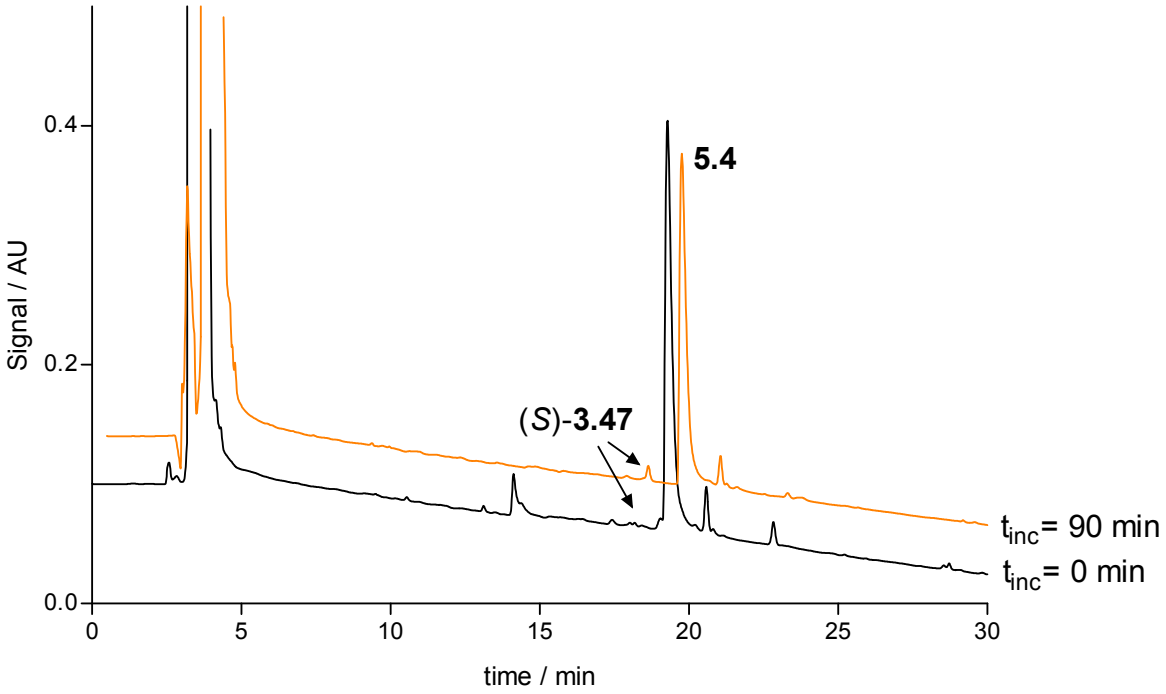


Figure 5.3. HPLC analysis of the Py-1 labeled fluorescent Y_2R antagonist **5.4** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

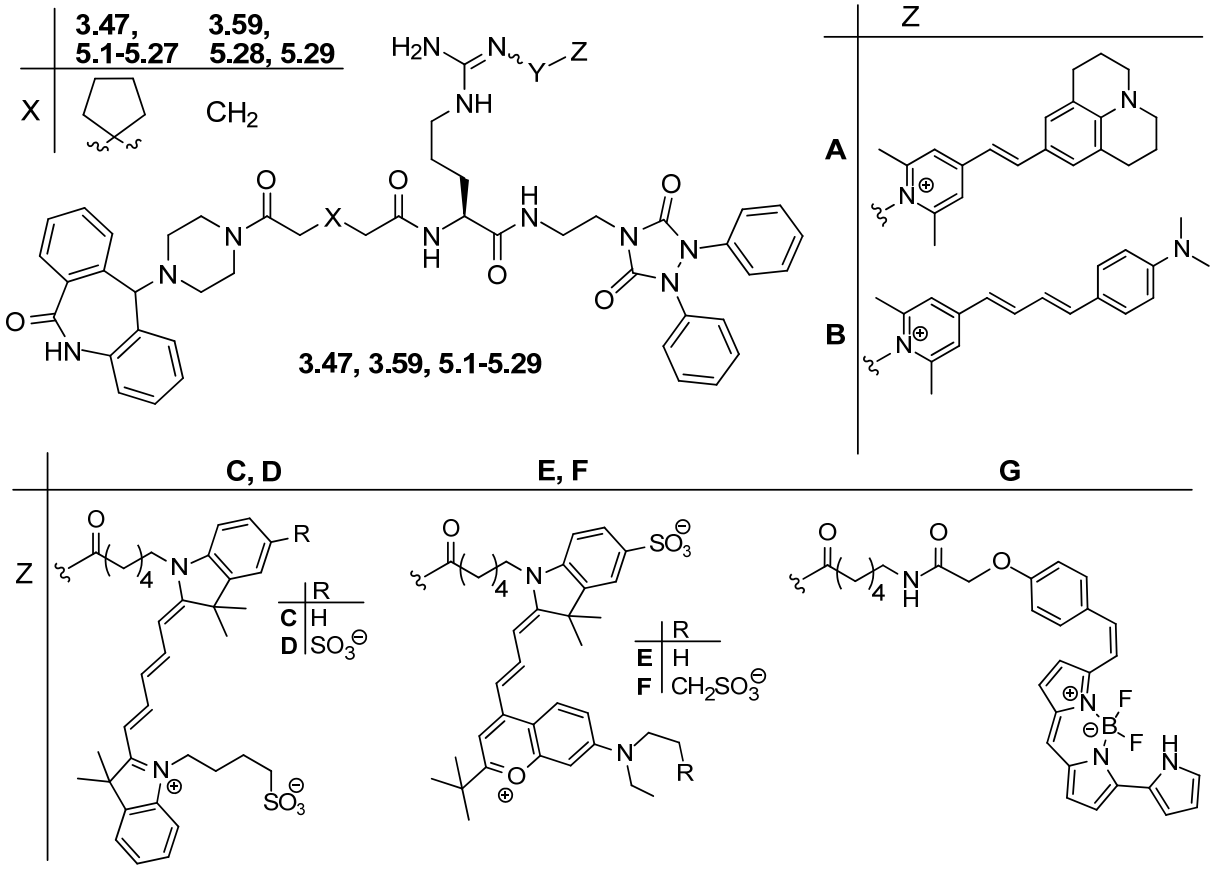
5.3 Pharmacological Results and Discussion

5.3.1 Pharmacology: Y_2R Antagonism, Affinity and Selectivity

The fluorescent Y_2R antagonists were investigated for Y_2R antagonism in a spectrofluorimetric Ca^{2+} assay (fura-2 assay) on CHO-hY_2R cells. The calcium indicator dye fura-2 is excited at 340 and 380 nm and the fluorescence intensity is measured at 510 nm. Hence, the spectroscopic properties of the fluorescence ligands do not interfere with the excitation and emission wavelengths of the fura-2-Ca^{2+} complex. Furthermore, binding of the Py-1 and Py-5 labeled antagonists **5.1**-**5.14** and **5.28** at the Y_2R was determined in a flow cytometric binding assay using Cy5-pNPY or Dy-635-pNPY. All other fluorescent ligands exhibit spectral properties similar to those of the applied labeled NPY derivatives. Thus, alternative standard ligands, excitable at wavelengths other than 635 nm (red diode laser), are needed to investigate these antagonists. Therefore, the Py-1 labeled compound **5.4** (excitable with an Ar laser at 488 nm) was used for the displacement with a selection of fluorescent Y_2R antagonists. Moreover, fluorescence ligands **5.4** and **5.16** were investigated in a radioligand binding assay displacing the novel Y_2R selective tritiated ligand [^{3}H]-UR-PLN196. The development and characterization of this radioligand is discussed in detail in Chapter 6. The results are summarized in Table 5.3.

The pyrylium-labeled ligands **5.1**-**5.14** proved to be highly potent antagonists at the Y_2R with K_i values in the range of the parent compound BIIE 0246. Only the Py-5 labeled compound **5.28** showed lower affinity than BIIE 0246 due to the alteration in the argininamide building block, but retained affinity referred to its guanidine analog (*S*)-**3.59**. In comparison to the appropriate amine precursors, the Py-labeling gave compounds with retained or slightly decreased affinity. Obviously, the introduction of the pyridinium fluorophores does not negatively affect binding and antagonistic activity because of the retained positive charge in the linker. Hence, a positive charge in the linker may contribute to Y_2 receptor binding by means of an additional interaction with the receptor as previously discussed. Lastly, the length of the linker seems to have a rather low impact on binding affinity.

Table 5.3. Structures, Y_2R antagonism and binding data of BIIE 0246 and fluorescent ligands **5.1**-**5.29**.



No	Y	Z	Spacer[a]	K_B / nM[b]	K_i / nM[c]
(*S*)-**3.47**[d]	H	--	--	5.6 ± 0.4	10.2 ± 1.1
5.1	$CO(CH_2)_3CONH(CH_2)_2$	A	8	4.6 ± 0.7	11 ± 1
5.2	$CO(CH_2)_3CONH(CH_2)_3$	A	9	1.3 ± 0.1	9.9 ± 0.1
5.3	$CO(CH_2)_3CONH(CH_2)_3$	B	9	1.9 ± 0.3	5.9 ± 0.3
5.4	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2$	A	11	9.8 ± 1.6	23 ± 4 73 ± 6[e]
5.5	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3$	A	18	3.8 ± 0.2	19 ± 5
5.6	$CONH(CH_2)_2$	A	4	3.2 ± 0.1	14 ± 7
5.7	$CONH(CH_2)_3$	A	5	4.3 ± 0.6	12 ± 0.6
5.8	$CONH(CH_2)_3$	B	5	4.0 ± 0.4	6.1 ± 0.2
5.9	$CONH(CH_2)_4$	A	6	6.4 ± 1.2	6.6 ± 0.7
5.10	$CONH(CH_2)_6$	B	8	7.2 ± 1.4	31 ± 15
5.11	$CO(CH_2)_5$	A	6	6.4 ± 0.2	26 ± 7
5.12	$CO(CH_2)_5$	B	6	3.3 ± 0.1	13 ± 2
5.13	$COCH_2(OCH_2CH_2)_2$	A	8	14 ± 3	8.5 ± 0.4
5.14	$CO(CH_2CH_2O)_4(CH_2)_2$	B	15	7.3 ± 1.6	12 ± 2
5.15	$CO(CH_2)_3CONH(CH_2)_3NH$	C	10	35 ± 7	300[f]
5.16	$CO(CH_2)_3CONH(CH_2)_3NH$	D	10	90 ± 70	982 ± 207[f] 614 ± 24[e]

Table 5.3 (continued)

5.17	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	C	12	14 ± 0.2	69[f]
5.18	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	D	12	12 ± 4	nd
5.19	$CO(CH_2)_2CONHCH_2(CH_2CH_2O)_3(CH_2)_3NH$	C	19	106 ± 34	nd
5.20	$CONH(CH_2)_2NH$	C	5	51 ± 35	nd
5.21	$CONH(CH_2)_3NH$	D	5	2714 ± 671	> 1000[f]
5.22	$COCH_2(OCH_2CH_2)_2NH$	C	9	259 ± 67	nd
5.23	$CO(CH_2)_3CONH(CH_2)_3NH$	F	10	663 ± 210	> 1000[f]
5.24	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	E	12	7.9 ± 3.6	nd
5.25	$CONH(CH_2)_6NH$	F	9	711 ± 203	> 1000[f]
5.26	$CO(CH_2)_3CONH(CH_2)_3NH$	G	10	nd	48[f]
5.27	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	G	12	63 ± 29	nd
(*S*)-**3.59**	H	--	--	28 ± 3	96 ± 13
5.28	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2$	B	11	11 ± 0.8	102 ± 2
5.29	$CO(CH_2)_3CONH(CH_2)_2NH(CH_2)_2NH$	C	12	70 ± 11	nd

[a] Length of the linker between the argininamide and the fluorescent dye given as number of atoms. [b] Inhibition of 70 nM pNPY induced $[Ca^{2+}]_i$ mobilization at CHO-hY_2-K9-qi5-K9-mtAEQ-A7 cells expressing the human Y_2R; mean values ± SEM (n = 2-4). [c] Flow cytometric binding assay using 5 nM Cy5-pNPY, or 10 nM Dy-635-pNPY as standard ligand at CHO-hY_2-K9-qi5-K9-mtAEQ-A7 cells; mean values ± SEM (n = 2-3). [d] BIIE 0246. [e] Displacement of [^{3}H]-UR-PLN196 (K_D = 65 nM; c = 75 nM) in a radioligand binding assay at CHO-hY_2-K9-qi5-K9-mtAEQ-A7 cells; mean values ± SEM (n = 2-3). [f] **5.4** (K_D = 24 nM; c = 40 nM) used as fluorescent ligand in flow cytometric competition binding; mean values ± SEM (n = 2-3).

Insertion of the higher molecular weight (hemi-)cyanine and Bodipy dyes by acylation of the amine precursors yielded fluorescent ligands devoid of a positive charge in the linker. This modification led to a drastic decrease in both potency and binding affinity of most of these compounds, apparently, because of the lack of a positive charge and the steric demand of the bulky fluorophore in proximity to the pharmacophoric moiety. Furthermore, S0387 and Dy-633 labeled fluorescent ligands **5.16**, **5.21**, **5.23** and **5.25** were inferior compared to the S0436 and Dy-630 coupled analogs. Strikingly, these ligands have one structural motif in common, namely an additional SO_3^- group in the fluorophore (for structures see Figure 5.1 and Table 5.3, respectively). When the pharmacophoric moiety of the fluorescent ligand is binding to the Y_2R, it is very likely that the fluorophores are oriented towards the extracellular region of the receptor protein due to lack of sufficient space between transmembrane domains. The extracellular loops of the Y_2R contain numerous acidic (negatively charged) amino acids.[33-34] Thus, the additional SO_3^- moiety could exhibit an electrostatic repulsion. Moreover, the sterical hindrance has to be taken into account. The drop in affinity proved to be less pronounced for antagonists **5.17**, **5.18**, **5.24** and **5.26**. Interestingly, these four fluorescent ligands have the same acyl linker, which includes a secondary amine function, in common. This observation once more underlines the affinity-enhancing effect of a positive charge in the linker.

The Y_2R selectivity was exemplarily investigated for compound **5.9** and **5.10** in flow cytometric binding studies using Cy5-pNPY at cells stably expressing human Y_1, Y_4 or Y_5 receptors (Table 5.4). Labeling with pyrylium dyes did not result in an alteration in selectivity compared to the precursors. As already discussed, the selectivity of S0436, S0387, Dy-630, Dy-633 and "Bodipy" labeled ligands could not be determined by flow cytometry due to their excitability with the red diode laser. For these compounds appropriate radiolabeled standard ligands have to be used.

Table 5.4. NPY receptor subtype selectivity of the fluorescent ligands **5.9** (Py-1 labeled) and **5.10** (Py-5 labeled).

No	K_i / nM[a]			
	Y_2R	Y_1R	Y_4R	Y_5R
5.9	6.6	> 350	> 6500	> 4500
5.10	31	> 3500	> 6500	> 4500

[a] Flow cytometric binding assays using 10 nM Cy5-pNPY (Y_1R), 5 nM Cy5-pNPY (Y_2R, Y_5R) or 3 nM Cy5-[K^4]-hPP (Y_4R) in HEL-Y_1 cells, CHO-Y_2 cells, CHO-Y_4 cells and HEC-1B-Y_5 cells, respectively.

5.3.2 Application of Fluorescent Y_2R Antagonists to Confocal Laser Scanning Microscopy

Various fluorescent Y_2R antagonists were screened for their applicability in confocal laser scanning microscopy on CHO cells expressing the hY_2R. The non-specific binding was determined in the presence of the non-fluorescent Y_2R selective antagonist BIIE 0246 in 100-fold excess. As shown in Figure 5.4 (panel A) the Py-1 labeled antagonist **5.2** is predominantly localized inside the cells. Fluorescence of the ligands was not detected in the nucleus. The uptake into the cells occurs within a few minutes even at very low concentrations (panel A2), presumably due to passive diffusion of the very lipophilic compound. Receptor internalization seems very unlikely, as antagonists have not been reported to cause internalization and down-regulation of GPCRs. The Py-1 labeled ligand **5.4** with an additional positive charge in the linker and concomitant reduced lipophilicity showed higher specific binding at the Y_2R (Figure 5.4 panel B).

Among the Py-labeled antagonists compound **5.28**, the Py-5 coupled analog of **5.4** lacking the cyclopentyl moiety in the argininamide pharmacophore, was found most suitable. Thus, the lower lipophilicity resulted in reduced diffusion through the cell membrane (Figure 5.4 panel C).

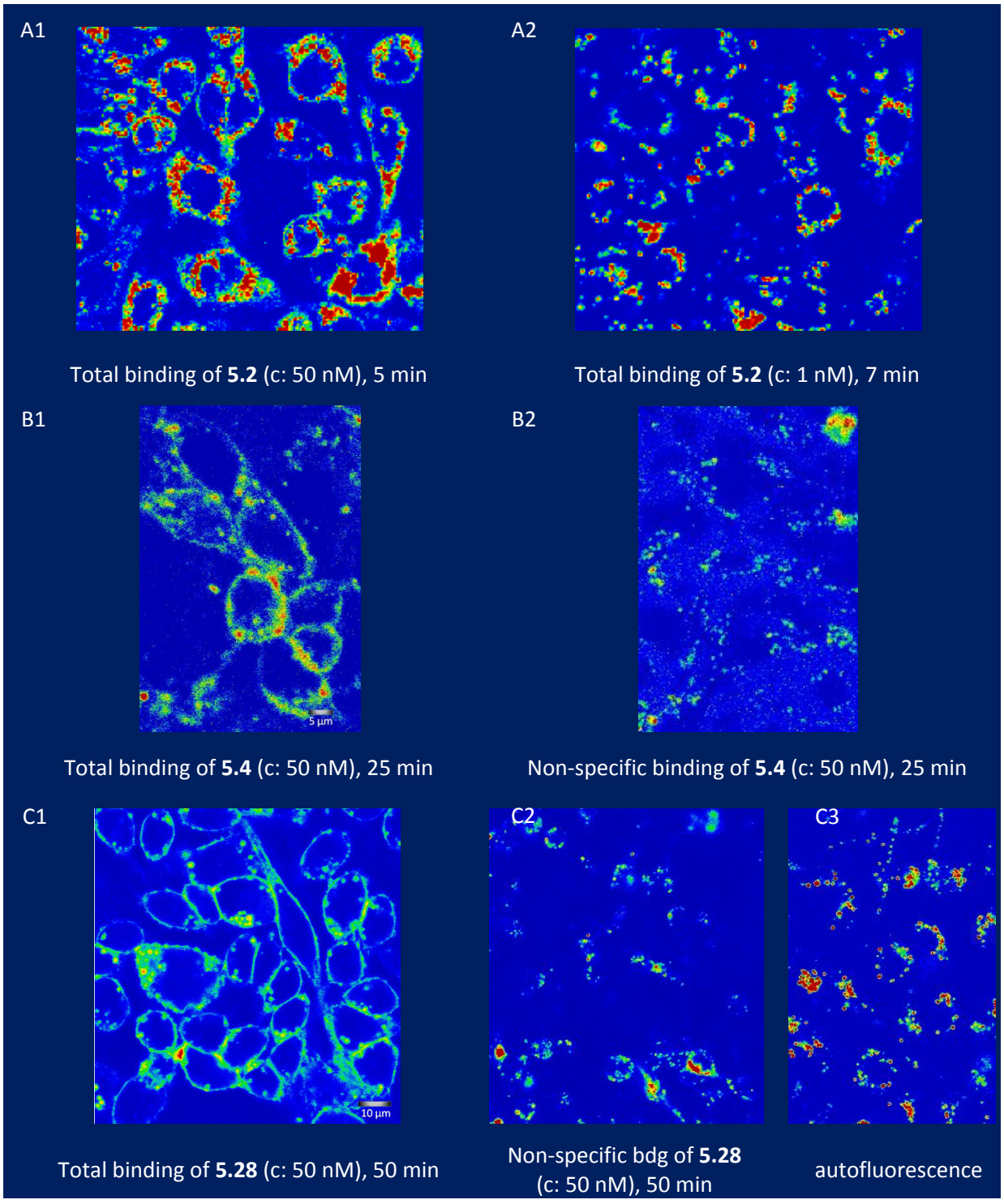


Figure 5.4. Binding of the fluorescent ligands **5.2** (panel A), **5.4** (panel B), **5.28** (panel C) to Y_2R expressed in CHO-hY_2R cells, visualized by confocal microscopy. Non-specific binding was determined in the presence of BIIE 0246 at 100-fold excess. Cells were incubated with the fluorescent ligands at rt in Leibowitz L15 culture medium. All images were acquired with a Zeiss Axiovert 200 M microscope.

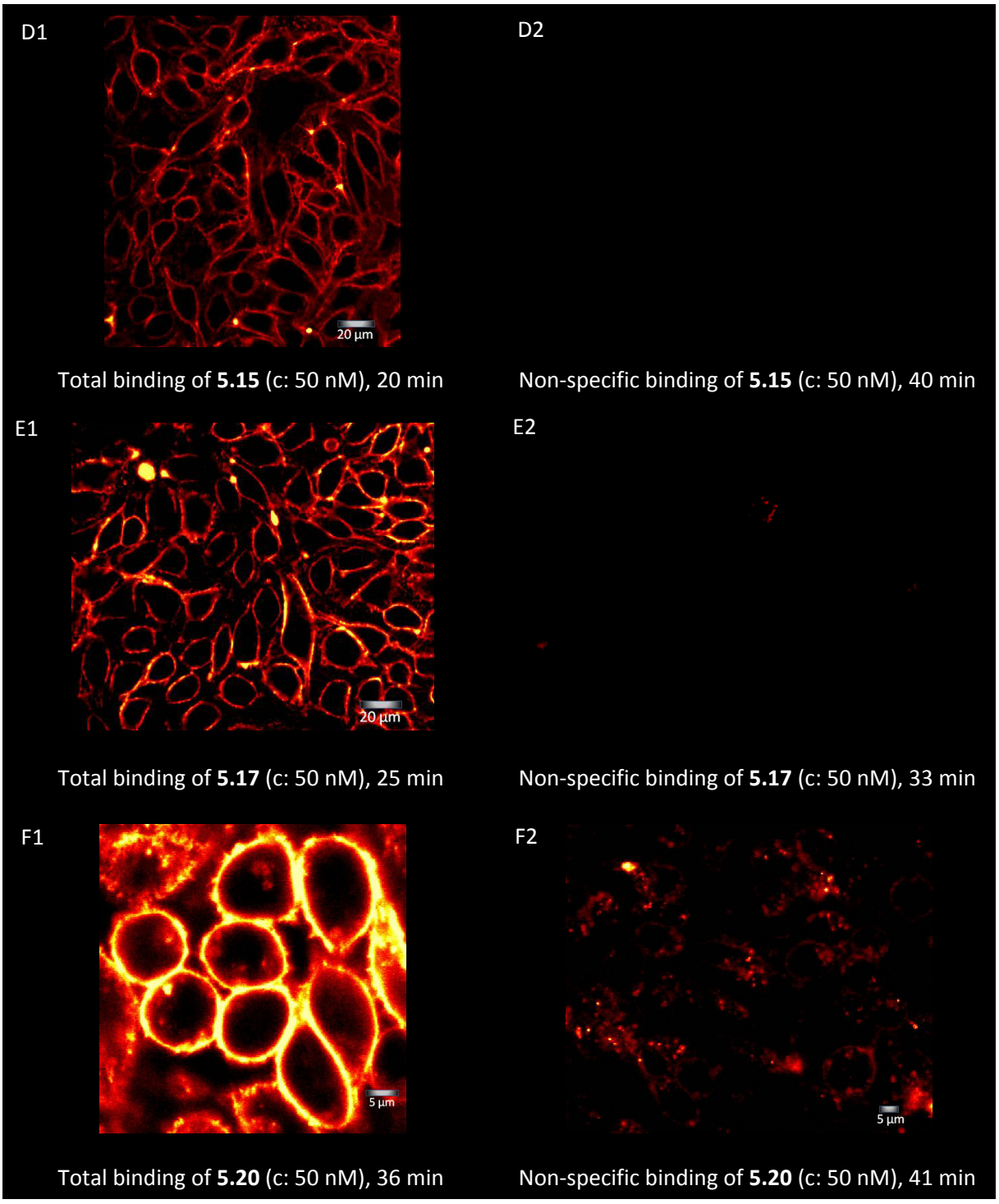


Figure 5.5. Binding of the fluorescent ligands **5.15** (panel D), **5.17** (panel E), **5.20** (panel F) to Y_2R expressed in CHO-hY_2R cells, visualized by confocal microscopy. Non-specific binding was determined in the presence of BIIE 0246 at 100-fold excess. Cells were incubated with the fluorescent ligands at rt in Leibowitz L15 culture medium. All images were acquired with a Zeiss Axiovert 200 M microscope.

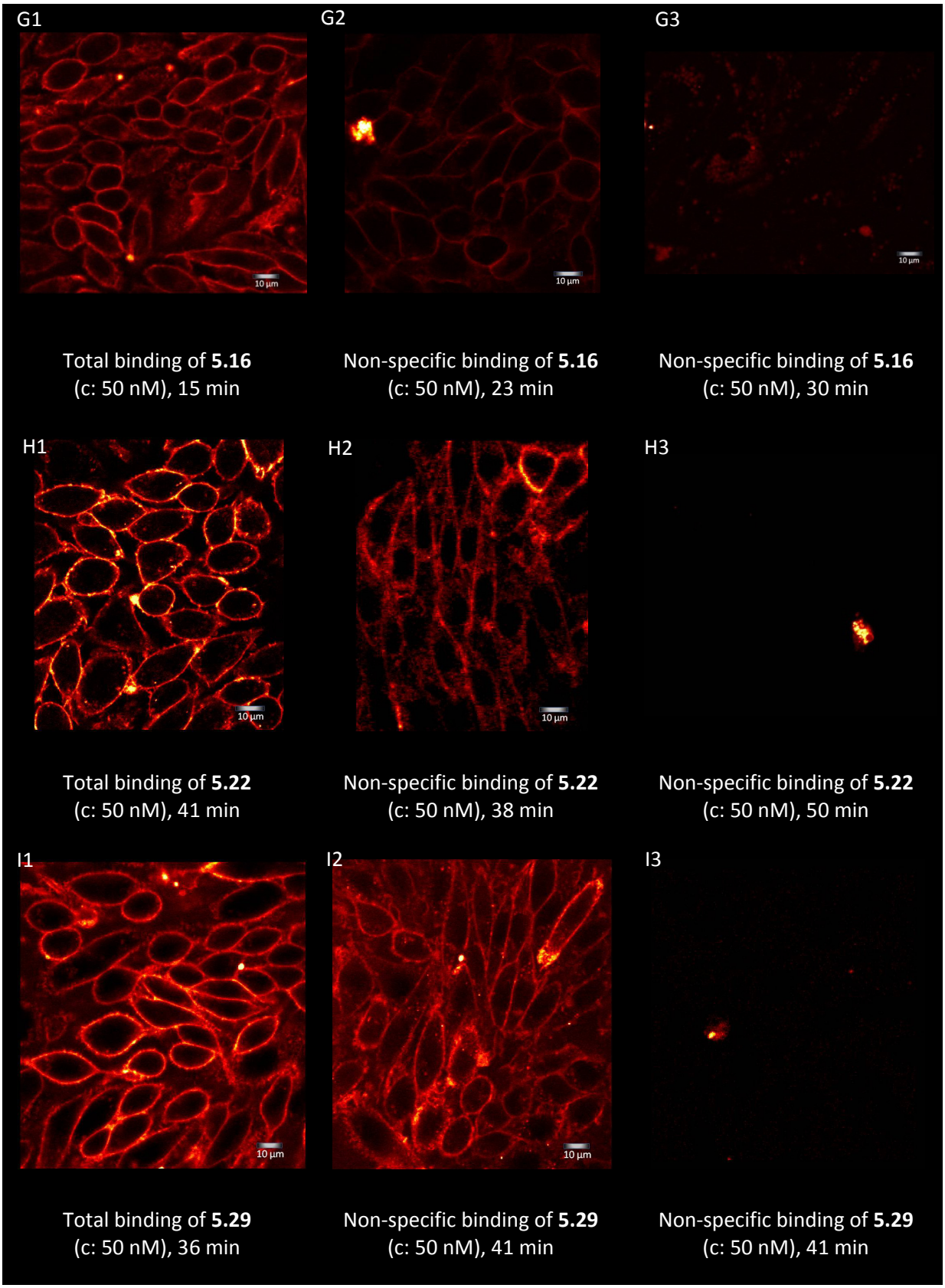


Figure 5.6. Binding of the fluorescent ligands **5.16** (panel G), **5.22** (panel H), **5.29** (panel I) to Y_2R expressed in CHO-hY_2R cells, visualized by confocal microscopy. 1: Total binding; 2: Displacement with pNPY at 500-fold excess; 3: Non-specific binding determined in the presence of BIIE 0246 at 100-fold excess. Cells were incubated with the fluorescent ligands at rt in Leibowitz L15 culture medium. All images were acquired with a Zeiss Axiovert 200 M microscope.

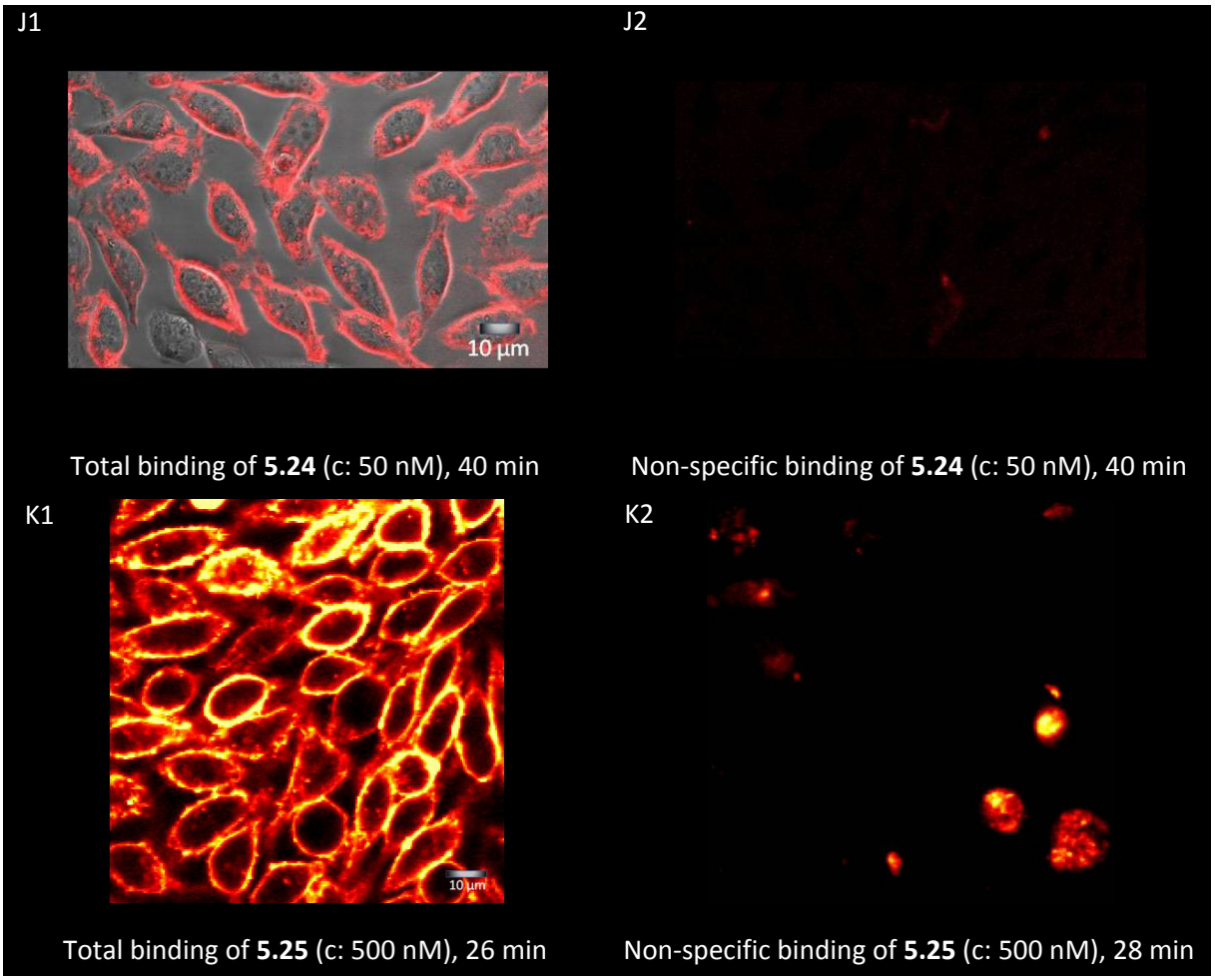


Figure 5.7. Binding of the fluorescent ligands **5.24** (panel J) and **5.25** (panel K) to Y_2R expressed in CHO-hY_2R cells, visualized by confocal microscopy. J1: Total binding merged; J2: Non-specific binding determined in the presence of BIIE 0246 at 100-fold excess (fluorescence image); K1: Total binding; K2: Non-specific binding determined in the presence of BIIE 0246 at 100-fold excess (fluorescence image). Cells were incubated with the fluorescent ligands at rt in Leibowitz L15 culture medium. All images were acquired with a Zeiss Axiovert 200 M microscope.

The S0436 and S0387 labeled fluorescent ligands which were applied to confocal laser scanning microscopy showed high specific binding to the Y_2R at a concentration as low as 50 nM (Figure 5.5 and Figure 5.6). Even compounds **5.16** and **5.22**, which are rather weak Y_2R antagonists (K_B values: 90 nM and 259 nM, respectively), revealed intense fluorescence in the membrane (Figure 5.6 panel G and H). Moreover, binding to the Y_2R seems to occur quite fast, as a high fluorescence signal was already observed after 15 min of incubation (Figure 5.6 G1). Non-specific binding was detected mainly inside the cells (cf. Figure 5.5 F2 and Figure 5.6 G3). However, penetration through the cell membrane was considerably reduced in comparison to the pyrylium labeled ligands.

Displacement of the fluorescent ligands **5.16**, **5.22** and **5.29** was performed with the endogenous ligand pNPY at a 500-fold excess – initially, in order to distinguish total and non-specific binding (Figure 5.6 panels G2, H2, I2). Surprisingly, the Y_2R antagonists were hardly displaced by pNPY. Interestingly, BIIE 0246 was reported to exhibit an insurmountable antagonism against NPY after pre-incubation with the cells

for 20 min, whereas it behaved as a competitive antagonist when co-incubated in a functional Ca^{2+} assay.[35] Nevertheless, in the case of the fluorescent ligands the poor displacement was observed despite co-incubation with pNPY. Thus, with respect to an explanation of this unusual behavior, more detailed investigations of the binding mode of argininamide-type Y_2R antagonists are urgently needed.
Furthermore, the Dy-630 and Dy-633 fluorophore containing antagonists **5.23**-**5.25** (data shown for **5.24** and **5.25** in Figure 5.7, panel J and K) showed high specific binding at the Y_2R with binding characteristics similar to S0436 and S0387 labeled fluorescent ligands. Binding was detectable at different concentrations corresponding to the antagonistic activities of the fluorescent ligands (cf. Table 5.3). While a 50 nM concentration was sufficient for the detection of the highly potent fluorescent ligand **5.24** (Figure 5.7 panel J1), membranes could only be visualized at a 500 nM concentration in case of the weak antagonist **5.25** (Figure 5.7 panel K1).

5.3.3 Fluorescent Y_2R Antagonists in Flow Cytometry: Kinetics, Saturation and Competition Binding Experiments

Several fluorescent Y_2R antagonists were chosen for more detailed investigations in flow cytometric binding experiments due to suited properties in terms of binding affinity, antagonistic activity, fluorescence properties and applicability in confocal microscopy. All experiments were performed on CHO-hY_2R cells.

5.3.3.1 Saturation Binding Experiments

To optimize the assay conditions, saturation binding experiments were performed with compounds **5.15** and **5.17** at different incubation periods using BSA containing or BSA free binding buffer. There was a significant increase in the ratio of total to non-specific binding when BSA free buffer was used (Table 5.5). Presumably, the increase in fluorescence intensity in the receptor bound state, resulting from rigidization, is more pronounced in the absence of BSA, compared to BSA containing binding buffer. Consequently, the following experiments were performed with BSA free binding buffer.

Four fluorescent Y_2R antagonists were chosen for saturation binding experiments (**5.4**, **5.15**-**5.17**). Besides compound **5.17**, all investigated fluorescent ligands showed very low non-specific binding even at high concentrations (Figure 5.8 and Figure 5.9). The saturation binding of the Py-1 labeled compound **5.4** was measured in the fluorescence channels Fl-2 (585 ± 21 nm) and Fl-3 (> 670 nm) and K_D values of 25 nM and 27 nM, respectively, were obtained (Figure 5.8). These data are in excellent agreement with the binding affinity determined by the displacement of Cy5-pNPY (K_i = 23 nM).

Table 5.5. Preliminary investigation on total (T) and non-specific (NS) binding of **5.15** (c = 25 nM; K_B = 35 nM). Influence of incubation time (t_{inc}) and BSA on receptor binding. NS was determined with 5 µM BIIE 0246.

t_{inc} /min	addition of BSA[a]	Ratio T / NS
15	+	3.9
15	-	9.3
30	+	3.8
30	-	8.1

[a] +: Addition of 1 % BSA to binding buffer (pH = 7.4); -: No BSA in the binding buffer (pH =7.4).

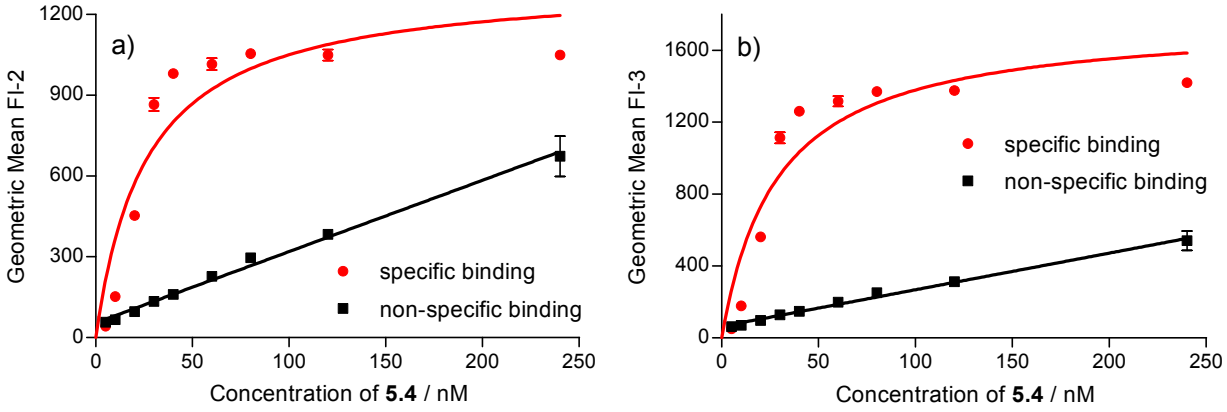


Figure 5.8. Flow cytometric saturation binding experiment with fluorescent ligand **5.4** at CHO-hY_2R cells (mean values ± SEM, n = 2, experiments performed in triplicate). Non-specific binding was determined in the presence of BIIE 0246 (100-fold excess); incubation time: 30 min. a) Measurement in Fl-2 (585 ± 21 nm); b) Measurement in Fl-3 (> 670 nm).

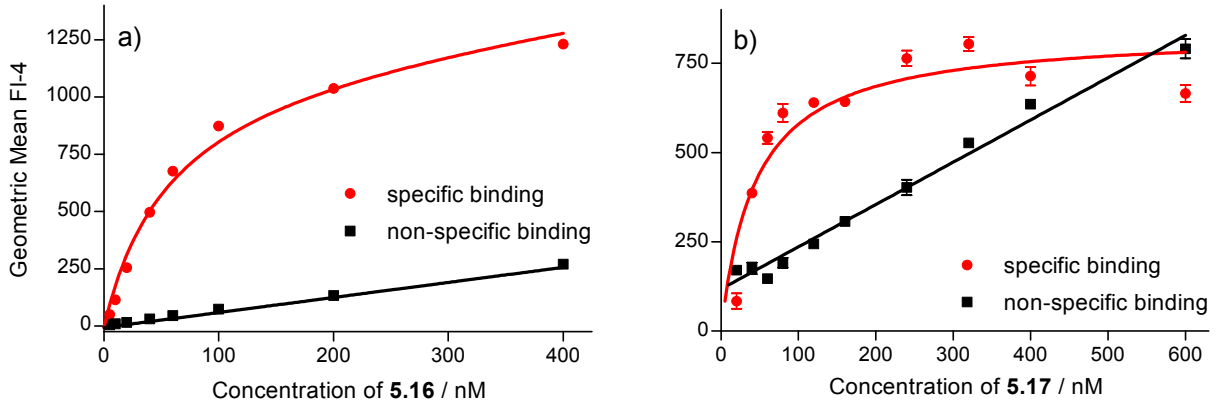


Figure 5.9. Flow cytometric saturation binding experiment with fluorescent ligands **5.16** and **5.17** at CHO-hY_2R cells (mean values ± SEM, n = 2, experiments performed in triplicate). Non-specific binding was determined in the presence of BIIE 0246 (100-fold excess); incubation time: 30 min; measurement in Fl-4 (661 ± 8 nm). a) Saturation binding of **5.16**. b) Saturation binding of **5.17**.

In case of the S0436 and S0387 labeled ligands **5.15**-**5.17** the fluorescence channel Fl-4 (661 ± 8 nm) was used for the determination of the dissociation constants. All ligands exhibited high to moderate binding affinity with K_D values of 42-143 nM (Table 5.6). It is noteworthy, that the S0387 labeled ligand **5.16** showed much higher binding affinity in saturation binding than expected from competition binding studies with the fluorescent ligand **5.4** and the radioligand [^{3}H]-UR-PLN196, and from the functional fura-2 assay, respectively (cf. Table 5.3 and Table 5.6, respectively).

In summary, the fluorescent ligands **5.4** and **5.15**-**5.17** revealed satisfactory results in saturation binding experiments, and therefore, were used in flow cytometric competition binding studies. Especially the Py-1 labeled ligand **5.4**, excitable by the argon laser (λ = 488 nm), and the S0387 labeled antagonist **5.16**, excitable by the red diode laser (λ = 635nm), showed favorable properties in terms of specific binding and affinity. Therefore, both compounds were selected for more detailed binding studies.

Table 5.6. Equilibrium dissociation constants of the fluorescent ligands **5.4**, **5.15**-**5.17**.

No	Saturation binding K_D / nM[a]
5.4	25 ± 1.4[b]
	27 ± 2[c]
5.15	143 ± 16
5.16	62 ± 5
5.17	42 ± 3

[a] Equilibrium dissociation constant determined on CHO-hY_2R cells, measured in Fl-4 (mean values ± SEM, n = 2, performed in triplicate). [b] Measurement in Fl-2. [c] Measurement in Fl-3.

5.3.3.2 Association and Dissociation Kinetics

The investigation of the association and the dissociation of compounds **5.4** and **5.16** should provide more detailed information about the binding properties of argininamide-type Y_2R antagonists. Therefore, the on- and off-rate (k_{on}, k_{off}), the half-life ($t_{1/2}$) and the kinetically derived equilibrium dissociation constant were determined.

The data of **5.16** are summarized in Table 5.7. The association reaches 90 % of maximum binding within around 30 minutes (Figure 5.10a), i. e., the association rate is rather slow in comparison to other non-peptidic antagonists. However, the fluorescent antagonist showed faster association than Cy5-pNPY, which reaches 90 % of maximum binding after about 110 minutes[36]. Interestingly, the dissociation of **5.16** remained incomplete even after 60 minutes (≈ 40 % specifically bound; Figure 5.10b).

Table 5.7. Y_2R binding characteristics of **5.16**.

k_{off} / min^{-1} [a]	k_{on} / min^{-1} · nM^{-1} [b]	k_{off}/k_{on} / nM[c]	K_D / nM[d]
0.01285	0.000526	24	62

[a] Dissociation rate constant determined by linear regression. [b] Association rate constant determined by linear regression. [c] Kinetically derived dissociation constant. [d] Equilibrium dissociation constant determined in saturation binding experiments.

Apparently, slow dissociation resulted in pseudo-irreversible binding. However, the dissociation rate of **5.16** was sufficient to calculate the kinetically derived K_D (k_{off}/k_{on} = 24 nM) by linearization of the association and dissociation curves (Figure 5.10c, d). This value is 2.5-times lower than the K_D received from saturation binding experiments (62 nM; Figure 5.9a). Yet, both values are in the same range. Taking the incomplete displacement of **5.16** into account, the calculated values can only be considered as an approximation of the actual half-lives ($t_{1/2}$) and off/on rates.[37] These findings prompted us to investigate the dissociation of the fluorescent labeled endogenous ligand Cy5-pNPY, a high affinity fluorescent ligand, which has been characterized in our workgroup in detail.[10, 38] In contrast to **5.16** the Cy5 labeled peptide revealed complete and relatively fast dissociation (Figure 5.11). 90 % of Cy5-pNPY were dissociated from the receptor after 60 minutes. Thus, the calculated K_D from kinetic studies is in good agreement with the dissociation constant from saturation binding (K_D (kinetic) = 6.1 nM; K_D (saturation) = 5.2 nM[10]) proving that the binding of the fluorescent peptide follows the law of mass action.[39]

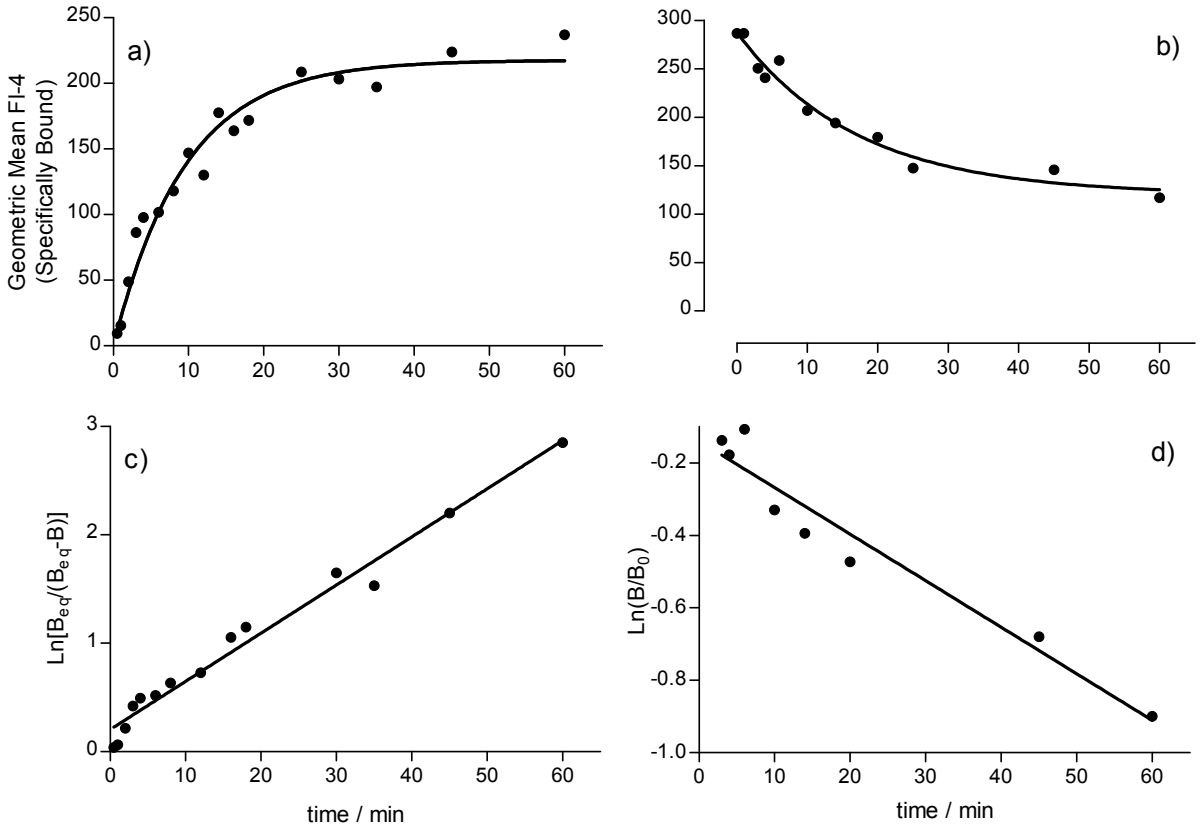


Figure 5.10. Binding kinetics of **5.16** at CHO-hY_2R cells. a) Association of **5.16** (c = 60 nM) as a function of time. b) Dissociation kinetics of **5.16** (pre-incubation: 60 nM, 30 min) in the presence of BIIE 0246 (c = 6 µM), monophasic exponential decay, $t_{1/2}$ = 11.9 min. c) Linearization of the association ($\ln[B_{eq}/(B_{eq}-B)]$ vs. time) for the determination of the on-rate (k_{on}): slope = k_{ob} = 0.04442 min^{-1}, k_{on} = (k_{ob} - k_{off})/[L] = 0.000526 $min^{-1} \cdot nM^{-1}$. d) Linearization of the dissociation ($\ln(B/B_0)$ vs. time) for the determination of the off-rate (k_{off}): slope (-1) = k_{off} = 0.01285 min^{-1}. (n = 3).

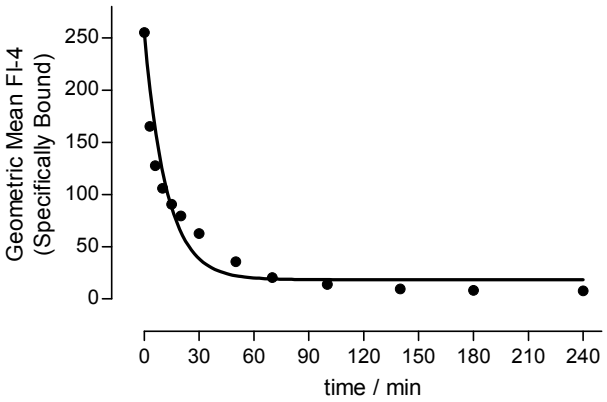


Figure 5.11. Dissociation of Cy5-pNPY (c = 5 nM) from the Y_2R as a function of time in the presence of pNPY (c = 500 nM), monophasic exponential decay, $t_{1/2}$ = 11.2 min.

For the Py-1 labeled fluorescent ligand **5.4** a time-dependent association similar to that of **5.16** was observed (Figure 5.12). Surprisingly, the dissociation experiments failed. There was no displacement in the presence of 100-fold excess of BIIE 0246, although the same concentration of BIIE 0246 was sufficient to determine the non-specific binding of **5.4** in saturation binding assays (cf. Figure 5.8). The critical difference between the assay procedures is the co-incubation of the fluorescent ligand with BIIE 0246 in case of saturation binding, whereas for dissociation experiments the fluorescent ligand is initially pre-incubated for 30 min with the cells, followed by a washing step and the addition of BIIE 0246 to the cell suspension. Thus, BIIE 0246 is unable to displace **5.4** when the latter has been pre-incubated with the cells. In principle, such a behavior could implement a distinct binding site or covalent binding of **5.4** to the receptor. However, both scenarios are highly unlikely. Both compounds share the same pharmacophoric moiety, irreversible binding of the Py-1 labeled fluorescent ligand was not observed when **5.4** was displaced with BIIE 0246 in saturation and competition binding experiments, and **5.4** does not possess reactive groups prone to covalent binding.

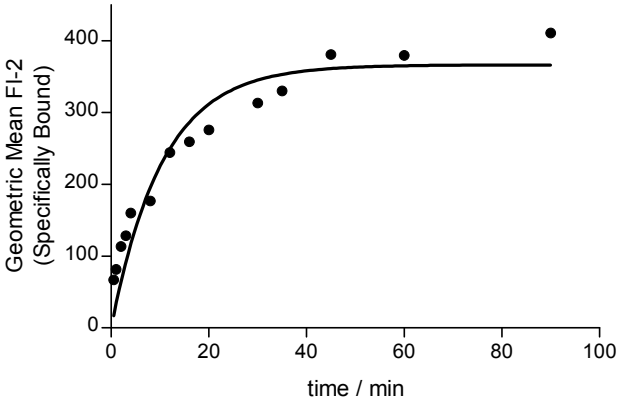


Figure 5.12. Association kinetic of **5.4** (c = 40 nM). Non-specific binding was determined with 100-fold excess of BIIE 0246.

5.3.3.3 Competition Binding Experiments

The applicability of selected fluorescent ligands (**5.4**, **5.15**-**5.17**) as reference compounds for the determination of Y_2R binding affinity was explored in competition binding experiments using several reference Y_2R ligands and new compounds described in this work. The fluorescent compounds were used at concentrations around their K_D values determined by saturation, taking into consideration the large difference between specific and non-specific binding at the respective concentrations, resulting in a high signal-to-noise ratio. The standard antagonist BIIE 0246 was used in equilibrium competition binding experiments with all four fluorescent ligands. Furthermore, the endogenous ligand pNPY, the potential radioligand (*S*)-**3.63**, and the recently developed small molecule Y_2R antagonist SF-11 were used for the displacement of the Py-1 labeled antagonist **5.4** and the S0387 coupled analog **5.16**. Unfortunately, SF-11 exhibited low binding affinities in the µM range at the Y_2R, despite moderate antagonism demonstrated in functional assays (IC_{50} = 199 nM[40]; IC_{50} (fura-2) = 349 nM; K_B = 77 nM). The calculated K_i values are summarized in Table 5.8.

As already observed in confocal microscopy, the high affinity agonist pNPY failed to displace the fluorescent antagonists (Figure 5.13). By contrast, the argininamide-type Y_2R fluorescent ligands were able to displace Cy5-pNPY in flow cytometric competition binding with high affinities (e.g., **5.4**: K_i = 23 nM). Presumably, interaction of pNPY with the agonist binding pocket is prevented by the antagonist priorly bound to the Y_2R. Thus, BIIE 0246-type ligands act as insurmountable (non-competitive) antagonists versus the endogenous agonist, probably due to distinct or at least not totally overlapping binding sites, or because of the stabilization of a ligand (antagonist)-specific receptor conformation. However, the calculated K_i values of BIIE 0246 and (*S*)-**3.63** are in good agreement with data from flow cytometric competition binding using Cy5-pNPY as standard ligand (Table 5.8).

Hence, the fluorescent antagonists are useful pharmacological tools in flow cytometric binding experiments for the characterization of non-peptidic antagonists. Yet, the characterization of peptide ligands could result in “false negatives” due to insurmountable ((pseudo)-irreversible) antagonism (Figure 5.13).

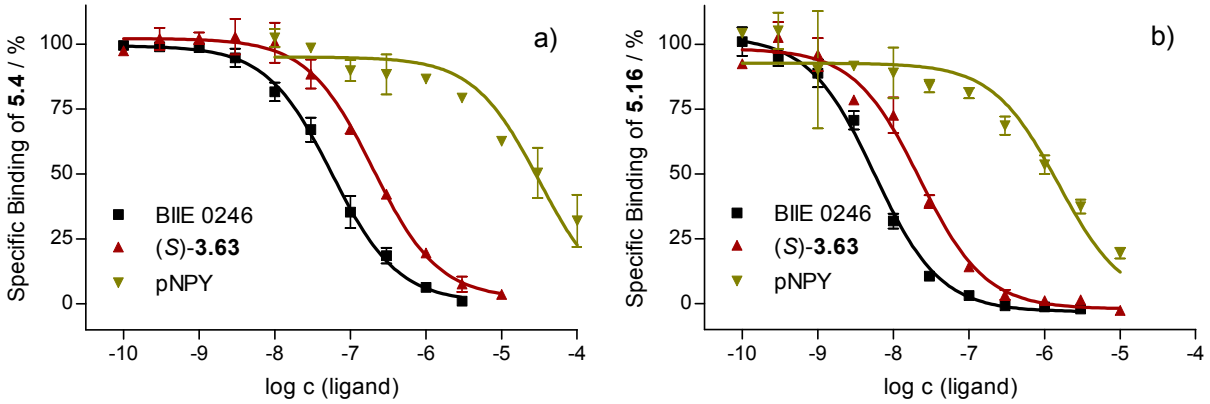


Figure 5.13. Flow cytometric competition binding on CHO-hY_2R cells; incubation time: 30 min for non-peptidic antagonists; incubation time: 90 min for pNPY; non-specific binding was determined with BIIE 0246 (c = 10 µM); mean values ± SEM (n = 2-5). a) Displacement of **5.4** (c = 40 nM, K_D = 25 nM) measured in Fl-2. b) Displacement of **5.16** (c = 60 nM, K_D = 60 nM) measured in Fl-4.

Table 5.8. K_i values determined in flow cytometric competition binding assays on CHO-hY_2R cells.

Compd.	Flow cytometric competition binding assay, K_i / nM displacement of				
	5.4[a]	**5.16**[b]	**5.15**[c]	**5.17**[d]	Cy5-pNPY[e]
BIIE 0246	22 ± 6	2.9 ± 0.3	19 ± 4	15 ± 1	10.2 ± 1.1
pNPY	12040 ± 1009	770 ± 190	nd	nd	0.8 ± 0.2[10]
(*S*)-**3.63**	77 ± 3	11 ± 0.6	nd	nd	9.9 ± 1.0[f]
SF-11	4915 ± 836	2890 ± 938	nd	nd	1251 ± 348

[a] K_D = 25 nM (Fl-2), c = 40 nM. [b] K_D = 62 nM (Fl-4), c = 60 nM. [c] K_D = 143 nM (Fl-4), c = 100 nM. [d] K_D = 42 nM (Fl-4), c = 40 nM. [e] K_D = 5.2 nM (Fl-4), c = 5 nM. [f] Determined with Dy-635-pNPY. (mean values ± SEM, n = 2-5).

5.4 Summary and Conclusion

The design of fluorescent Y_2R ligands was based on the application of the guanidine-acylguanidine bioisosteric approach to the argininamide-type Y_2R antagonist BIIE 0246. Herein, the guanidine group was linked to fluorophores *via* ω-aminoacyl spacers of different lengths and chemical nature. The fluorescent label was attached to the primary amines by acylation with succinimidyl esters or by ring transformation of pyrylium dyes. The majority of the synthesized derivatives proved to be potent and selective fluorescence labeled Y_2R antagonists. However, the binding affinity strongly depended on the chemical nature of the fluorescent dye.

The low molecular weight pyrylium dyes turned out to be well suited for fluorescence labeling of Y_2R antagonists exhibiting retained affinity compared to the parent compound BIIE 0246. Hence, a preserved positive charge in the ω-aminoacyl spacer after ring transformation is assumed to be favorable in terms of receptor binding, as the positively charged resonance-stabilized pyridinium may contribute to Y_2R binding by electrostatic interactions. The attachment of bulky dyes by acylation and concomitant elimination of the positive charge led to a more or less pronounced

decrease in activity compared to the parent argininamide. As expected, a preserved positive charge in the linker was favorable in terms of antagonistic activity (**5.17**, **5.18**, **5.24**). By contrast, S0387 and Dy-633 labeled compounds (**5.16**, **5.21**, **5.23**, **5.25**), comprising a SO_3^- moiety in the fluorophore, appeared to be inferior compared to their S0436 and Dy-630 coupled analogs (**5.15**, **5.20**), presumably due to electrostatic repulsion with extracellular loop regions of the receptor.

The majority of the cyanine (S0436, S0387), Dy-630 and Dy-633 labeled fluorescent ligands were successfully applied in confocal microscopy even at very low concentrations relative to their K_B and K_i values (cf. **5.16**). In case of pyrylium coupled ligands, **5.4** and **5.28** revealed suitable binding properties, whereas rapid diffusion into the cells was observed for the Py-1 and Py-5 fluorescent antagonists **5.1**-**5.3**, **5.5**-**5.14**. A selection of fluorescent ligands turned out to be appropriate for investigations in flow cytometric equilibrium binding studies. The versatility of this application makes fluorescence-labeled Y_2R antagonists a promising alternative to the commonly used universal NPY receptor ligand Cy5-pNPY.

In addition, the novel fluorescent probes were utilized in more detailed studies on the binding mode of argininamide-type Y_2R antagonists. In contrast to Cy5-pNPY, kinetic experiments with the fluorescent ligand **5.16** revealed pseudo-irreversible binding. Furthermore, an insurmountable binding versus the natural ligand pNPY was observed for several fluorescent antagonists in flow cytometric competition binding (**5.4**, **5.16**) and confocal microscopy (**5.16**, **5.22**, **5.29**), respectively.

In summary, the new fluorescent probes can be regarded as useful reference compounds and pharmacological tools for fluorescence-based screening and detailed pharmacological characterization of small molecule Y_2R ligands. Moreover, these fluorescent ligands enable the optical detection of NPY Y_2 receptors *in vitro* as demonstrated on hY_2R expressing CHO cells.

5.5 Experimental Section

5.5.1 General Experimental Conditions

Chemicals and solvents were purchased from commercial suppliers and used without further purification. Pyrylium dyes Py-1 and Py-5 (tetrafluoroborate salts) were kindly provided by the Institute of Analytical Chemistry, Chemo- and Biosensors at the University of Regensburg (Prof. Dr. O. S. Wolfbeis). These dyes are also commercially available from Active Motif Chromeon (www.activemotif.com). The succinimidyl esters of fluorescent dyes S0436 and S0387 were obtained from FEW Chemicals (Bitterfeld-Wolfen, Germany). The succinimidyl esters of fluorescent dyes Dy-630 and Dy-633 were obtained from Dyomics (Jena, Germany). The Bodipy 650/665-X succinimidyl ester (SE) and Cy5-NHS ester were obtained from Molecular Probes (now Invitrogen; Darmstadt, Germany) and GE Healthcare LifeSciences (Freiburg,

Germany), respectively. Porcine NPY (pNPY) was kindly provided by the Institute of Organic Chemistry I at the Ruhr-University Bochum (Prof. Dr. C. Cabrele). SF-11 was purchased from Tocris Bioscience (Bristol, United Kingdom). All solvents were of analytical grade or distilled prior to use. DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH.

Low resolution mass spectrometry analysis (MS) was performed in-house on a Finnigan ThermoQuest TSQ 7000 (ES-MS) and a Finnigan SSQ 710A (EI-MS 70 eV, CI-MS). High resolution mass spectrometry analysis (HRMS) for compound **5.5** was performed on a JMS-700 instrument (JOEL Ltd, Tokyo, Japan). Lyophilization was done with a Christ alpha 2-4 LD equipped with a vacuubrand RZ 6 rotary vane vacuum pump.

Preparative HPLC was performed on a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector and two RP-columns (Nucleodur 100-5 C18ec, 250 × 21 mm, 5 µm; Gemini-NX C18, 250 × 21 mm, 5 µm, AXIA Packed) at a flow rate of 22 mL/min. Mixtures of acetonitrile and 0.1 % aq. TFA were used as mobile phase. Acetonitrile was removed from the eluates under reduced pressure (final pressure: 90 mbar) at 45 °C prior to lyophilization. Analytical HPLC analysis was performed on two different systems (Thermo Separation Products, Merck) which are listed in detail below. Mixtures of acetonitrile (A) and 0.05 % aq. TFA (B) (Thermo Separation Products), and mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B) (Merck), respectively, were used as mobile phase. Helium degassing was used throughout.

Stock solutions (2 or 10 mM) of all compounds were prepared in DMSO and stored at –20 °C.

Analytical HPLC systems:

System 1: Thermo Separation Products; composed of a SN400 controller, a P4000 pump, a degasser (Degassex DG-4400, phenomenex), an AS3000 autosampler and a Spectra Focus UV-VIS detector; flow rate: 0.8 mL/min; UV detection: 220 nm.

System 2: Merck; composed of a L-5000 controller, a 655A-12 pump, a 655A-40 autosampler and a L-4250 UV-VIS detector; flow rate: 0.7 mL/min; UV detection: 220 nm.

Applied gradients:

Gradient 1 (Thermo Separation Products): 0 to 30 min: A/B 20/80 to 95/5, 30 to 37 min: 95/5.

Gradient 2 (Merck): 0 to 30 min: A/B 20/80 to 95/5, 30 to 40 min: 95/5.

The purity of all compounds was determined by HPLC using system 1 (gradient 1) on a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany), except for compounds **5.18**, **5.24** and **5.27**, which were measured with a Nucleodur H-Tec 100-5 C18 column (250 × 4 mm, 5 µm, Macherey-Nagel, Düren, Germany). HPLC system 2

(gradient 2) was used for reaction monitoring on a Eurospher-100 C18 column (250 × 4 mm, 5 µm, Knauer, Berlin, Germany).

5.5.2 Chemistry: Experimental Protocols and Analytical Data

General procedure for the preparation of Py-1 and Py-5 labeled fluorescent ligands 5.1-5.14, 5.28:

The pertinent amine precursor (2-4 eq) and NEt_3 (8-15 eq) were dissolved in a mixture of CH_3CN and DMF (≈ 10 % DMF v/v, total volume: 400-800 µL) followed by the addition of the pyrylium dye × BF_4^- (1 eq) in DMF (≈ 50 µL). The reaction was stopped by addition of 10 % aq. TFA (corresponding to 6-10 eq TFA) after an incubation period of 1-3 h at rt. The reaction mixture was adjusted with 0.1 % aq. TFA to a volume of ≈ 2-4 mL resulting in a ratio of CH_3CN/0.1 % aq. TFA, which approximately corresponds to the ratio of the solvents CH_3CN/0.1 % TFA in the starting eluent (max. two times higher concentration of CH_3CN). The reaction mixtures were subjected to preparative HPLC yielding the products as red solids after lyophilization.

1-((15*S*)-10-Amino-19-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,8,16-trioxo-15-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,9,11,17-tetraazanonadec-9-enyl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.1). Yield: 52 % (1.73 mg); MS (ES, +p) *m/z* (%): 671 (100) $[M+H]^{2+}$; RP-HPLC: 92 % (t_R = 22.7 min, k' = 7.4); $C_{77}H_{91}N_{14}O_8^+ \times C_2F_3O_2^-$ (1425.70).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12,16-trioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,9,11,17-tetraazaicos-10-en-20-yl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.2). Yield: 35 % (1.20 mg); MS (ES, +p) *m/z* (%): 678 (100) $[M+H]^{2+}$; RP-HPLC: 94 % (t_R = 22.9 min, k' = 7.5); $C_{78}H_{93}N_{14}O_8^+ \times C_2F_3O_2^-$ (1466.72).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12,16-trioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,9,11,17-tetraazaicos-10-en-20-yl)-4-{4-[4-(dimethyl-amino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.3). Yield: 36 % (1.59 mg); MS (ES, +p) *m/z* (%): 665 (100) $[M+H]^{2+}$, 443 (32) $[M+2H]^{3+}$; RP-HPLC: 96 % (t_R = 20.8 min, k' = 6.7); $C_{76}H_{91}N_{14}O_8^+ \times C_2F_3O_2^-$ (1440.70).

1-((18*S*)-13-Amino-22-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-7,11,19-trioxo-18-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,6,12,14,20-pentaazadocos-12-enyl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.4). Yield: 39 % (1.79 mg); MS (ES, +p) *m/z* (%): 692 (30) $[M+H]^{2+}$, 462 (100) $[M+2H]^{3+}$; RP-HPLC: 90 % (t_R = 18.3 min, k' = 5.8); $C_{79}H_{96}N_{15}O_8^+ \times C_2F_3O_2^-$ (1495.75).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12,15-trioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}ethyl)cyclopentyl]acetamido}-20,23,26-trioxa-3,9,11,16-tetraazanonacos-10-en-29-yl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.5). Yield: 50 % (2.25 mg); MS (ES, +p) *m/z* (%): 744 (100) $[M+H]^{2+}$; HRMS (LSI-MS): *m/z* $[M+H]^+$ calcd. for $C_{84}H_{106}N_{14}O_{11}^+$: 1486.8160, found: 1486.8101; RP-HPLC: 62 % (t_R = 23.9 min, k' = 7.9); $C_{84}H_{105}N_{14}O_{11}^+ \times C_2F_3O_2^-$ (1598.80).

1-((11*S*)-6-Amino-15-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12-dioxo-11-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,5,7,13-tetraazapentadec-5-enyl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.6). Yield: 49 % (1.86 mg); MS (ES, +p) *m/z* (%): 636 (100) $[M+H]^{2+}$; RP-HPLC: 64 % (t_R = 22.8 min, k' = 7.4); $C_{73}H_{85}N_{14}O_7^+ \times C_2F_3O_2^-$ (1382.66).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12-dioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,9,11,13-tetraazahexadec-10-en-16-yl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.7). Yield: 45 % (1.68 mg); MS (ES, +p) *m/z* (%): 643 (100) $[M+H]^{2+}$; RP-HPLC: 61 % (t_R = 24.6 min, k' = 8.1); $C_{74}H_{87}N_{14}O_7^+ \times C_2F_3O_2^-$ (1396.68).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12-dioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,9,11,13-tetraazahexadec-10-en-16-yl)-4-{4-[4-(dimethylamino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.8). Yield: 17 % (0.73 mg); MS (ES, +p) *m/z* (%): 630 (100) $[M+H]^{2+}$; RP-HPLC: 98 % (t_R = 21.7 min, k' = 7.0); $C_{72}H_{85}N_{14}O_7^+ \times C_2F_3O_2^-$ (1370.66).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12-dioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,9,11,13-tetraazaheptadec-10-en-17-yl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.9). Yield: 35 % (1.52 mg); MS (ES, +p) *m/z* (%): 650 (100) $[M+H]^{2+}$; RP-HPLC: 66 % (t_R = 25.1 min, k' = 8.3); $C_{75}H_{89}N_{14}O_7^+ \times C_2F_3O_2^-$ (1410.69).

1-((5*S*)-10-Amino-1-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-4,12-dioxo-5-{2-[1-(2-oxo-2-{4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,9,11,13-tetraazanonadec-10-en-19-yl)-4-{4-[4-(dimethylamino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.10). Yield: 33 % (1.88 mg); MS (ES, +p) *m/z* (%): 651 (100) $[M+H]^{2+}$; RP-HPLC: 78 % (t_R = 23.2 min, k' = 7.6); $C_{75}H_{91}N_{14}O_7^+ \times C_2F_3O_2^-$ (1412.71).

1-{6-[Amino((4*S*)-5-{2-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]ethylamino}-5-oxo-4-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}ethyl)cyclopentyl]acetamido}pentylamino)methyleneamino]-6-oxohexyl}-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.11). Yield: 67 % (3.31 mg); MS (ES, +p) *m/z* (%): 649 (100) $[M+H]^{2+}$; RP-HPLC: 72 % (t_R = 24.0 min, k' = 7.9); $C_{76}H_{90}N_{13}O_7^+ \times C_2F_3O_2^-$ (1409.70).

1-{6-[Amino((4*S*)-5-{2-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]ethylamino}-5-oxo-4-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}ethyl)cyclopentyl]acetamido}pentylamino)methyleneamino]-6-oxohexyl}-4-{4-[4-(dimethylamino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.12). Yield: 36 % (1.25 mg); MS (ES, +p) *m/z* (%): 636 (100) $[M+H]^{2+}$; RP-HPLC: 98 % (t_R = 22.6 min, k' = 7.4); $C_{72}H_{84}N_{13}O_7^+ \times C_2F_3O_2^-$ (1355.65).

1-((15*S*)-10-Amino-19-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-8,16-dioxo-15-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}-ethyl)cyclopentyl]acetamido}-3,6-dioxa-9,11,17-triazanonadec-9-enyl)-4-[2-(1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-9-yl)vinyl]-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.13). Yield: 24 % (1.18 mg); MS (ES, +p) *m/z* (%): 665 (100) $[M+H]^{2+}$; RP-HPLC: 90 % (t_R = 24.1 min, k' = 7.9); $C_{76}H_{90}N_{13}O_9^+ \times C_2F_3O_2^-$ (1441.69).

1-((22*S*)-17-Amino-26-[3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl]-15,23-dioxo-22-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,6,9,12-tetraoxa-16,18,24-triazahexacos-16-enyl)-4-{4-[4-(dimethylamino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.14). Yield: 23 % (0.90 mg); MS (ES, +p) *m/z* (%): 703 (100) $[M+H]^{2+}$; RP-HPLC: 90 % (t_R = 22.0 min, k' = 7.1); $C_{79}H_{98}N_{13}O_{11}^+ \times C_2F_3O_2^-$ (1517.74).

1-((18*S*)-13-Amino-18-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-carbamoyl]-7,11,20,24-tetraoxo-24-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}-3,6,12,14,19-pentaazatetracos-12-enyl)-4-{4-[4-(dimethyl-amino)phenyl]buta-1,3-dienyl}-2,6-dimethylpyridinium 2,2,2-trifluoroacetate (5.28). Yield: 15 % (0.75 mg); MS (ES, +p) *m/z* (%): 652 (75) $[M+H]^{2+}$, 435 (100) $[M+2H]^{3+}$; RP-HPLC: 94 % (t_R = 15.2 min, k' = 4.6); $C_{73}H_{88}N_{15}O_8^+ \times C_2F_3O_2^-$ (1415.68).

General procedure for the preparation of S0436, S0387, Dy-630, Dy-633, and Bodipy 650/665-X labeled fluorescent ligands 5.15-5.27, 5.29:

The pertinent amine precursor (2 eq) and NEt_3 (8 eq) were dissolved in CH_3CN (200-400 µL), followed by the addition of the dye-NHS ester (1 eq) in CH_3CN (100-200 µL) and DMF resulting in a total volume of 300-600 µL. For the acylation with S0387-NHS ester ≈ 10-20 % DMF v/v was added to the CH_3CN solution prior to addition to the stirred reaction mixture. The reaction was stopped by addition of 10 % aq. TFA (corresponding to 6-10 eq TFA) after an incubation period of 8-16 h at rt. The reaction mixture was adjusted to a volume of ≈ 2-4 mL by addition of 0.1 % aq. TFA resulting in a ratio of CH_3CN/0.1 % aq. TFA, which approximately corresponds to the ratio of the solvents (CH_3CN/0.1 % TFA) in the starting eluent (max. two times higher concentration of CH_3CN). The reaction mixtures were subjected to preparative HPLC yielding the products as cyan or blue solids after lyophilization.

4-[2-(5-{1-[(5*S*)-10-Amino-1-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-4,12,16,22-tetraoxo-5-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,9,11,17,21-pentaazaheptacos-10-en-27-yl]-3,3-dimethylindolin-2-ylidene}penta-1,3-dienyl)-1,1-dimethyl-1*H*-iso-indolium-3-yl]butane-1-sulfonate 2,2,2-trifluoroacetate (5.15). Yield: 73 % (2.88 mg); MS (ES, +p) *m/z* (%): 1654 (40) $[M+H]^+$, 827 (100) $[M+2H]^{2+}$; RP-HPLC: 92 % (t_R = 24.3 min, k' = 8.0); $C_{92}H_{113}N_{12}O_{12}S \times C_2HF_3O_2$ (1765.83).

Compound (*S*)-3.49 labeled with S0387 (5.16). Yield: 23 % (0.56 mg); MS (ES, +p) *m/z* (%): 868 (100) $[M+3H]^{2+}$; RP-HPLC: 99 % (t_R = 16.5 min, k' = 5.1); $C_{92}H_{112}N_{15}O_{15}S_2^- \times C_4H_2F_6O_4$ (1958.77).

Compound (*S*)-3.50 labeled with S0436 (5.17). Yield: 58 % (1.54 mg); MS (ES, +p) *m/z* (%): 842 (25) $[M+2H]^{2+}$, 562 (100) $[M+3H]^{3+}$; RP-HPLC: 97 % (t_R = 20.2 min, k' = 6.5); $C_{93}H_{116}N_{16}O_{12}S \times C_2HF_3O_2$ (1794.86).

Compound (*S*)-3.50 labeled with S0387 (5.18). Yield: 34 % (1.20 mg); MS (ES, +p) *m/z* (%): 882 (100) $[M+3H]^{2+}$; RP-HPLC: 99 % (t_R = 12.5 min, k' = 4.4); $C_{93}H_{115}N_{16}O_{12}S_2^- \times C_4H_2F_6O_4$ (1987.80).

Compound (*S*)-3.51 labeled with S0436 (5.19). Yield: 83 % (1.83 mg); MS (ES, +p) *m/z* (%): 1786 (30) $[M+H]^+$, 894 (100) $[M+2H]^{2+}$; RP-HPLC: 87 % (t_R = 23.9 min, k' = 7.9); $C_{98}H_{125}N_{15}O_{15}S \times C_2HF_3O_2$ (1897.91).

Compound (*S*)-3.52 labeled with S0436 (5.20). Yield: 65 % (1.54 mg); MS (ES, +p) *m/z* (%): 785 (100) $[M+2H]^{2+}$; RP-HPLC: 97 % (t_R = 24.9 min, k' = 8.2); $C_{87}H_{105}N_{15}O_{11}S \times C_2HF_3O_2$ (1681.77).

Compound (*S*)-3.53 labeled with S0387 (5.21). Yield: 43 % (1.05 mg); MS (ES, +p) *m/z* (%): 832 (100) $[M+3H]^{2+}$; RP-HPLC: 97 % (t_R = 17.5 min, k' = 5.5); $C_{87}H_{105}N_{15}O_{11}S^- \times C_4H_2F_6O_4$ (1795.76).

Compound (*S*)-3.57 labeled with S0436 (5.22). Yield: 29 % (1.84 mg); MS (ES, +p) *m/z* (%): 1628 (20) $[M+H]^+$, 815 (100) $[M+2H]^{2+}$; RP-HPLC: 93 % (t_R = 24.5 min, k' = 8.1); $C_{90}H_{110}N_{14}O_{13}S \times C_2HF_3O_2$ (1740.80).

Compound (*S*)-3.49 labeled with Dy-633 (5.23). Yield: 73 % (0.81 mg); MS (ES, +p) *m/z* (%): 889 (100) $[M+3H]^{2+}$; RP-HPLC: 98 % (t_R = 18.8 min, k' = 6.0); $C_{94}H_{116}N_{15}O_{16}S_2^- \times C_4H_2F_6O_4$ (2002.80).

1-[(5*S*)-10-Amino-1-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-4,12,16,24-tetraoxo-5-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,9,11,17,20,23-hexaazanonacos-10-en-29-yl]-2-{3-[2-tert-butyl-7-(diethylamino)chromenylium-4-yl]allylidene}-3,3-dimethylindoline-5-sulfonate 2,2,2-trifluoroacetate (5.24). Yield: 34 % (0.93 mg); MS (ES, +p) *m/z* (%): 857 (35) $[M+2H]^{2+}$, 572 (100) $[M+3H]^{3+}$; RP-HPLC: 100 % (t_R = 15.0 min, k' = 5.5); $C_{94}H_{118}N_{16}O_{13}S \times C_2HF_3O_2$ (1824.87).

Compound (*S*)-3.55 labeled with Dy-633 (5.25). Yield: 23 % (0.26 mg); MS (ES, +p) *m/z* (%): 875 (100) $[M+3H]^{2+}$; RP-HPLC: 93 % (t_R = 22.0 min, k' = 7.1); $C_{93}H_{116}N_{15}O_{15}S_2^- \times C_4H_2F_6O_4$ (1974.80).

3-{4-[(5*S*)-10-Amino-1-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-4,12,16,22,29-pentaoxo-5-{2-[1-(2-oxo-2-{4-[6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl]-piperazin-1-yl}ethyl)cyclopentyl]acetamido}-3,9,11,17,21,28-hexaazatriacont-10-en-30-yloxy]styryl}-5,5-difluoro-7-(1*H*-pyrrol-2-yl)-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*]-[1,3,2]diazaborinin-4-ium-5-uide 2,2,2-trifluoroacetate (5.26). Yield: 40 % (0.60 mg); MS (ES, +p) *m/z* (%): 798 (100) $[M+2H]^{2+}$; RP-HPLC: 98 % (t_R = 25.6 min, k' = 8.5); $C_{86}H_{98}BF_2N_{17}O_{11}$ (1593.77).

Compound (*S*)-3.50 labeled with Bodipy 650/665-X (5.27). Yield: 56 % (1.27 mg); MS (ES, +p) *m/z* (%): 812 (30) $[M+2H]^{2+}$, 542 (100) $[M+3H]^{3+}$; RP-HPLC: 87 % (t_R = 15.5 min, k' = 5.7); $C_{87}H_{102}BF_2N_{18}O_{11}$ (1623.80).

4-{2-[5-(1-{(7*S*)-12-Amino-7-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl-carbamoyl]-1,5,14,18,26-pentaoxo-1-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl]-6,11,13,19,22,25-hexaazahentriacont-12-en-31-yl}-3,3-dimethylindolin-2-ylidene)penta-1,3-dienyl]-3,3-dimethyl-3*H*-indolium-1-yl}butane-1-sulfonate 2,2,2-trifluoroacetate (5.29). Yield: 57 % (1.48 mg); MS (ES, +p) *m/z* (%): 815 (85) $[M+2H]^{2+}$, 544 (100) $[M+3H]^{3+}$; RP-HPLC: 87 % (t_R = 18.0 min, k' = 5.7); $C_{89}H_{110}N_{16}O_{12}S \times C_2HF_3O_2$ (1740.81).

5.5.3 Synthesis and Purification of Cy5-pNPY

Cy5-pNPY was synthesized as previously described.[38] HPLC purification was performed by analytical HPLC using system 2 equipped with a Jupiter 300 C18 column (250 × 4.6 mm, 5µm, Phenomenex, Aschaffenburg, Germany). Conditions: UV detection: 275 nm; fluorescence detection: λ_{ex} = 645 nm, λ_{em} = 670 nm; eluent: mixtures of acetonitrile + 0.025 % TFA (A) and 0.025 % aq. TFA (B), gradient: 0 to 30 min: A/B 25/75 to 35/65, 30 to 32 min: 35/65 to 95/5, 32 to 47 min: 95/5, t_R = 32.0 min (UV). After evaporation the product was re-diluted in CH_3CN/0.1 aq. TFA (20/80, v/v). The concentration was determined by UV/vis spectroscopy at 649 nm, using the molar extinction coefficient of cy5 for the appropriate solvent mixture determined previously by R. Ziemek (313568 $L \cdot mol^{-1} \cdot cm^{-1}$)[36]. Afterwards, the labeled peptide was aliquoted (1 nmol) into siliconized microtubes (1.5 mL) and the solvent was evaporated in the vacuum concentrator at room temperature. The purified product was analyzed by MS (MALDI-TOF: *m/z* = 4927).

5.5.4 Fluorescence Spectroscopy and Determination of Quantum Yields

The quantum yields were determined with a Cary Eclipse spectrofluorimeter and a Cary 100 UV/VIS photometer (Varian Inc., Mulgrave, Victoria, Australia). The photomultiplier voltage of the Cary Eclipse spectrofluorimeter was set to 400 V throughout. Recording of excitation spectra was performed with an excitation slit of 5 nm and an emission slit of 10 nm. Emission spectra depicted in Figure 5.2 were recorded with an excitation slit and an emission slit of 10 nm, respectively. The quantum yields were determined by analogy with a previously reported protocol[12] using cresyl violet perchlorate (Acros Organics, Geel, Belgium) as red fluorescent standard, for which a quantum yield of 54 % in ethanol was reported in the literature[41]. Determination of the spectra was performed in acryl cuvettes (10 × 10 mm, Ref. 67.755, Sarstedt, Nümbrecht, Germany). For fluorescence spectroscopy, solutions corresponding to absorbances between 0.08 and 0.2 at the respective

excitation wavelength were used. The appropriate concentration of the fluorescent ligands was determined by UV/vis spectroscopy; absorption spectra were recorded within a concentration range of 3-10 µM. For the pyrylium labeled compounds **5.1**-**5.14** the excitation wavelength was chosen as close to the absorption maximum as possible. The (hemi-)cyanine- and Bodipy-labeled ligands (**5.15**-**5.27**) were excited at a plateau of the absorption spectrum. It was strictly avoided to excite the fluorescent compounds in flank of the excitation spectrum.
Solutions of the fluorescent ligands were freshly prepared from 2 or 10 mM stock solutions of the compounds in DMSO and immediately protected from light. Fluorescence spectra were recorded at two different slit adjustments (excitation/emission): 10/5 nm, 10/10 nm. For the determination of reference spectra, the pure solvents with the same DMSO content were measured.
Instrument settings for the recorded emission spectra: T = 22 °C; `medium scan rate´; filter settings: auto (excitation filter), open (emission filter); $\lambda_{em} = \lambda_{ex}$ + 10-15 nm.
Substraction of the corresponding reference spectrum from every emission spectrum yielded the net spectra, which were multiplied with the appropriate lamp correction spectra, resulting in the corrected emission spectra (cf. Figure 5.2). The corrected emission spectra were integrated up to 850 nm.
The absorbance at the excitation wavelength was determined by recording absorption spectra immediately after recording the emission spectra (within 30 min after preparation of test solutions). Baselines were stored using reference solutions and substracted from the raw spectra. The quantum yield was calculated according to the following equation[41]:

$$\phi_{F(X)} = (A_s/A_x)\,(F_x/F_s)\,(n_x/n_s)^2\,\phi_{F(S)}$$

A: absorbance of the corrected absorption spectrum at the excitation wavelength;
F: integral of the corrected emission spectrum;
n: refraction index of the solvent;
ϕ_F: quantum yield / %;
s: cresyl violet perchlorate standard; $\phi_{F(S)} = 54$;
x: fluorescent ligand (sample).

5.5.5 Investigation of the Chemical Stability

The stability of the fluorescent Y_2R antagonists **5.4**, **5.10**, **5.14**, **5.17** and **5.22** regarding the formation of BIIE 0246 was investigated at neutral pH in FACS binding buffer (cf. Chapter 5.5.6.1). Incubation was started by addition of 10 µL of a 1 mM solution of the compounds in DMSO to 190 µL of buffer giving a final concentration of 50 µM. After 90 minutes incubation, a 80 µL aliquot was taken and diluted with a mixture of CH_3CN, H_2O and 1 % aq. TFA (5:1:4, 80 µL). 100 µL of the resulting solution (pH = 2-3) was analyzed by HPLC on a RP-column (Eurospher-100 C18, 250 × 4 mm, 5 µm, Knauer, Berlin, Germany) using a system from Thermo Separation Products

(composed of a SN400 controller, a P4000 pump, a degasser (Degassex DG-4400, phenomenex), an AS3000 autosampler and a Spectra Focus UV/vis detector). Mixtures of acetonitrile (A) and 0.05 % aq. TFA (B) were used as mobile phase (gradient: 0 to 30 min: A/B 20/80 to 95/5, 30 to 37 min: A/B 95/5). The flow rate was set to 0.8 mL/min, the column temperature to 30 °C and the detection wavelength to 220 nm. The quantity of decomposition product BIIE 0246 ((*S*)-**3.47**) was estimated from the peak integrals of the incubation sample and a reference chromatogram of the analyzed compound (incubation time: 0 min).

5.5.6 Receptor Binding and Functional Assays

5.5.6.1 Cell Culture and Buffers

Cell culture. CHO cells stably expressing the hY_2R were cultured as described elsewhere.[10]

Buffers. The buffer for the Y_2R binding studies on CHO cells was prepared by the addition of BSA (1 %) and bacitracin (100 $\mu g \cdot L^{-1}$) to a buffer (pH 7.4) consisting of HEPES (25 mM), $CaCl_2 \times 2\ H_2O$ (2.5 mM), $MgCl_2 \times 6\ H_2O$ (1 mM). BSA and bacitracin were omitted in saturation binding, kinetic and competition binding experiments with non-peptide fluorescent antagonists. For the displacement of **5.4** and **5.16** with pNPY only BSA was omitted. The loading buffer (pH 7.4) for the determination of the mobilization of intracellular Ca^{2+} in CHO cells was prepared by dissolving NaCl (120 mM), KCl (5 mM), $MgCl_2 \times 6\ H_2O$ (2 mM), $CaCl_2 \times 2\ H_2O$ (1.5 mM), HEPES (25 mM), and glucose (10 mM).

5.5.6.2 Fura-2 Calcium Assay and Radioligand Competition Binding Experiments

The fura-2 assay was performed as previously described using a LS50 B spectrofluorimeter from Perkin Elmer (Überlingen, Germany).[42] Radioligand competition binding experiments with [^{3}H]-UR-PLN196 were performed for the determination of K_i values of the fluorescent ligands **5.4** and **5.16**. as described in Chapter 6.

5.5.6.3 Flow Cytometry

All flow cytometric binding assays were performed with a FACSCalibur™ flow cytometer (Becton Dickinson, Heidelberg, Germany), equipped with an argon laser (488 nm) and a red diode laser (635 nm).[10, 38, 43] All binding assays were performed in a final volume of 500 µL containing labeled ligand and unlabeled peptide or antagonist as needed.

Selectivity assay. The selectivity of the new Y_2R antagonists for human NPY Y_2 over Y_1, Y_4 and Y_5 receptors was investigated by flow cytometric binding assays on Cy5-pNPY (Y_1R, Y_5R), and Cy5-[K^4]-hPP (Y_4R) according to previously described methods.[10, 38, 44] The compounds were tested at two concentrations (1 µM and 10 µM) in duplicate.

Competition binding assay with Cy5-pNPY. The binding assay was performed as described elsewhere[10] with the following adaptations. Instrument settings were: FSC: E-1, SSC: 350 V, Fl-4: 800 V, Flow: HI; measurement stopped when 10000 gated events were counted. The experiments were performed using 490 µL of cell suspension, 5 µL of Cy5-pNPY (final concentration 5 nM) and 5 µL of test compound (100-fold concentrated). If indicated, 5 µL of Dy-635-pNPY (final concentration 10 nM) was used as fluorescent ligand. Incubation time was 90 min at room temperature. K_i values were obtained from 2-3 independent experiments.

Displacement of fluorescent ligands 5.4, and 5.15-5.17. The cells were seeded three days prior to the experiment (90-95 % confluency), trypsinized and resuspended in Ham´s F12 medium, containing 10 % fetal calf serum for the inactivation of trypsin. After centrifugation at 1,000 rpm for 5 min, the cells were suspended in binding buffer without BSA to a density of 2-3 · 10^6 cells/mL. Bacitracin (0.1 g/L) was added in case of competition binding with pNPY in order to prevent protease-mediated degradation of the peptide. The experiments were performed with the respective fluorescent ligand (c (**5.4**) = 40 nM, c (**5.15**) = 100 nM, c (**5.16**) = 60 nM, c (**5.17**) = 40 nM) in the presence and absence of various competitors at different concentrations (10^{-10} to 10^{-4} M). Non-specific binding was determined in the presence of 10 µM BIIE 0246. The samples were incubated in siliconized reaction vessels for 30 min (90 min for pNPY) at room temperature before flow cytometer analysis. Instrument settings for fluorescent ligand **5.4**: FSC: E-1, SSC: 360 V, Fl-2: 550 V, Fl-3: 800 V, Flow: HI; measurement stopped when 10000 gated events were counted; Instrument settings for fluorescent ligands **5.15-5.17**: FSC: E-1, SSC: 350 V, Fl-4: 600 V, Flow: HI; measurement stopped when 10000 gated events were counted.

Saturation binding experiments with 5.4, and 5.15-5.17. Saturation binding experiments were performed by analogy with the above mentioned competition binding assays with following adaptations. The cells were incubated with the respective fluorescent ligand in increasing concentrations (c ≈ 0.2 × K_D - 10 × K_D) for 30 min. Non-specific binding was determined with 100-fold excess of BIIE 0246.

Kinetics of Y_2R binding of Cy5-pNPY, 5.4 and 5.16. The cells were treated as already described. Association kinetics was determined by incubation of the cells with a constant concentration of labeled ligand (c (Cy5-pNPY) = 5 nM, c (**5.4**) = 40 nM, c (**5.16**) = 60 nM). Samples were taken at different time periods and measured (For

instrument settings see the respective competition binding protocols). Non-specific binding was measured with 100-fold excess of pNPY in case of Cy5-pNPY and BIIE 0246 for the labeled non-peptide ligands, respectively.
For dissociation experiments cells were pre-incubated with the corresponding labeled ligand at the above mentioned concentrations for 30 min (Cy5-pNPY: 120 min) in binding buffer without BSA, bacitracin, except for Cy5-pNPY (buffer + 1 % BSA and 0.1 g/L bacitracin). For non-specific binding, pre-incubation was performed in presence of 100-fold excess of pNPY in case of Cy5-pNPY and BIIE 0246 for the non-peptide labeled ligands, respectively. Afterwards, cells were washed with cold binding buffer and resuspended in binding buffer consisting of pNPY and BIIE 0246 (100-fold excess), respectively. Samples were taken at different time periods and measured. (For instrument settings see the respective competition binding protocols).

5.5.6.4 Data Processing

All data to be fitted were processed with GraphPad Prism 5.

Fura-2 Assay. The ratio of fluorescence intensities at 510 nm after excitation at 340 and 380 nm was used for the calculation of the calcium concentration according to the Grynkiewicz equation.[45] At least two points between 20 and 80 % inhibition served for the calculation of IC_{50} values after logit-log transformation using the following equation:
logit (P) = log [P/(100-P)]
IC_{50} values were obtained by plotting logit (P) versus log B with the slope n according to logit (P) = $n$log B–$n$log IC_{50} by linear regression. The K_B value was calculated using the Cheng-Prusoff equation[46]:
$K_B = IC_{50} \cdot [EC_{50}/(EC_{50}+L)]$
P: mean percent inhibition with SEM < 10 %, determined from 2-3 independent experiments performed on different days;
B: antagonist concentration;
IC_{50}: concentration of competitor producing 50 % inhibition;
EC_{50}: agonist concentration that induces 50 % of the maximum response;
L: concentration of pNPY.

Radioligand Binding Assay. IC_{50} values were converted to K_i values according to the Cheng-Prusoff equation[46] using the respective K_D value of the radioligand.

Flow Cytometry. The geometric means of fluorescence in the corresponding fluorescence channel (Fl-2, Fl-3, Fl-4) of the gated cells were calculated using the WinMDI 2.9 software. The inhibition constant K_i was calculated using the Cheng-Prusoff equation[46]:

$K_i = IC_{50} \cdot [K_D/(K_D+L)]$

K_i: inhibition constant of the competitor;

K_D: dissociation constant of the labeled ligand;

IC_{50}: concentration of unlabeled competitor producing 50 % inhibition of the specific binding of the labeled ligand;

L: concentration of the labeled ligand.

Data from saturation binding were evaluated by one-site saturation fits. Rate constants (k_{ob}, k_{off}) were analyzed by linear regressions. The association rate constant (k_{on}) was calculated according to the following equation:

$k_{on} = (k_{ob}–k_{off})/L$

L: concentration of the ligand;

k_{ob}: observed/macroscopic association rate constant;

k_{off}: dissociation rate constant.

5.5.7 Confocal Microscopy

Two days prior to the experiment CHO-hY_2R cells were trypsinized and seeded in Nunc LabTek™ II chambered coverglasses with 8 chambers (Nunc, Wiesbaden, Germany), or in 1 µ-Slides 8 well ibiTreat sterile glasses (ibidi GmbH; München, Germany) in Ham´s F12 medium, containing 10 % fetal calf serum. On the day of the experiment confluency of the cells was 60-90 %. The culture medium was removed, the cells were washed twice with Leibowitz L15 culture medium (200 µL) and covered with L15 medium (120 µL). L15 medium (40 µL) and 5-fold concentrated fluorescent probe in L15 medium (40 µL) was added for total binding. For non-specific binding L15 medium (40 µL) with the competing agent BIIE 0246 (5-fold concentrated) and L15 medium (40 µL) with the fluorescent probe (5-fold concentrated) was added. Images of total and non-specific binding were acquired after an incubation period of 5-50 min.

Confocal microscopy was performed with a Zeiss Axiovert 200 M microscope, equipped with the LSM 510 laser scanner. The objective was a Plan-Apochromat 63x/1.4 with oil immersion. Table 5.9 shows the most important settings for the detection of the investigated fluorescent ligands.

Table 5.9. Conditions for the detection of the fluorescent ligands **5.2**, **5.4**, **5.15**-**5.17**, **5.20**, **5.22**-**5.26**, **5.28** and **5.29** with the Zeiss Axiovert 200 M microscope.

No	Excitation (laser transmission)	Filter	Pinhole / µm
5.2	514 nm (10 %)	LP 560	78
5.4	488 nm (10 %)	LP 560	276
5.15	633 nm (10 %)	LP 650	276
5.16	633 nm (10 %)	LP 650	276
5.17	633 nm (10 %)	LP 650	276
5.20	633 nm (10 %)	LP 650	276
5.22	633 nm (10 %)	LP 650	94
5.23	633 nm (10 %)	LP 650	276
5.24	633 nm (10 %)	LP 650	288
5.25	633 nm (10 %)	LP 650	276
5.26	633 nm (10 %)	LP 650	276
5.28	488 nm (10 %)	LP 650	276
5.29	633 nm (10 %)	LP 650	276

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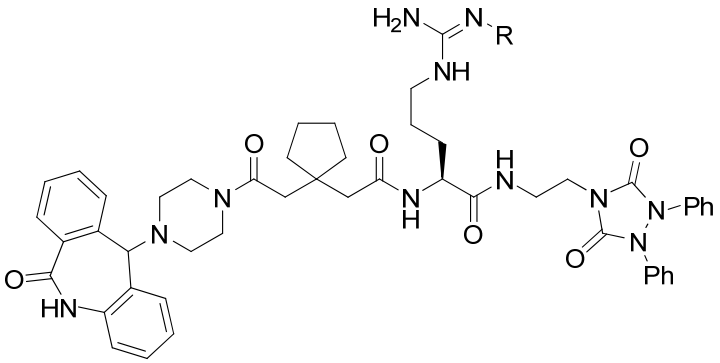
Chapter 6 [^{3}H]-UR-PLN196: A Highly Potent and Selective Tritiated Neuropeptide Y Y_2 Receptor Antagonist

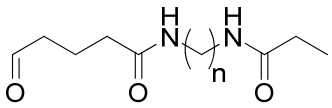
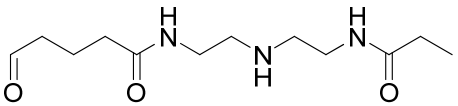
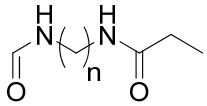
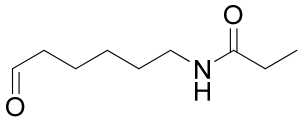
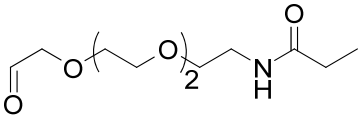
6.1 Introduction

Radioligands are frequently used as pharmacological tools to characterize new compounds with respect to their binding properties and their pharmacological profile. Such radioligands should be characterized by high purity (> 90 %), sufficient stability and high specific activity (> 10 Ci/mmol) in terms of chemical properties, as well as subtype selectivity, fast kinetics and appropriate mode of action (antagonism) with respect to the pharmacological profile. Concerning the Y_2R, the commonly applied standard ligands like [^{3}H]-NPY,[1-2] [^{125}I]-PYY[3] or [^{125}I]-PYY(3-36)[4-5] are not considered suitable for labeling, especially due to lacking selectivity. For instance, in autoradiography utilizing [^{125}I]-PYY(3-36), discrimination between BIIE 0246-sensitive and BIIE 0246-insensitive binding sites is required due to strong binding of the radioligand to the Y_5R sites (BIIE 0246-insensitive sites).[6] Hence, novel non-peptidic highly selective radiolabeled antagonists are required as pharmacological tools for more detailed investigations of the Y_2 receptor subtype.

Therefore, we prepared a series of derivatives of the highly potent and selective argininamide-type Y_2R antagonist BIIE 0246.[7] Previously, the parent compound was found to tolerate electron-withdrawing substituents attached to the guanidine group (N^G), including moieties comprising a terminal amino group (N^ω), in terms of Y_2R binding regardless of the lowered basicity by 4-5 orders of magnitude.[8] These findings prompted us to synthesize a small library of potential radioligands (“cold” versions) by propionylation of the terminal amino moiety, whereof an appropriate candidate was chosen for the development of a tritiated selective Y_2R antagonist (Table 6.1).

Table 6.1. Structures and Y_2R affinities of BIIE 0246, selected N^{ω}-substituted potential radioligands and the N^{G}-propionylated analog **3.86**.

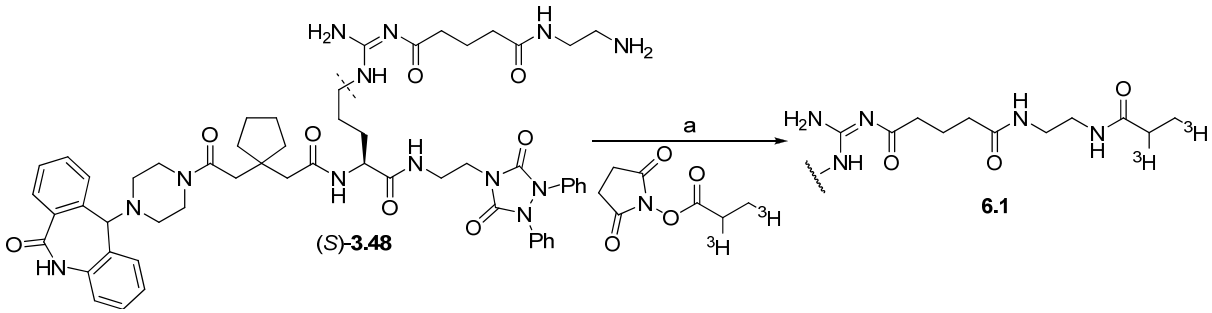


compound	R	n	K_i / nM[a]
BIIE 0246	H	--	10.2
(*S*)-**3.63**		2	9.9[b]
(*S*)-**3.64**		3	37[b]
(*S*)-**3.65**		--	9.9
(*S*)-**3.68**		3	32
(*S*)-**3.69**		4	55
(*S*)-**3.71**		--	9.0[b]
(*S*)-**3.72**		--	15
3.86		--	5.2

[a] Displacement of Cy5-pNPY in a flow cytometric competition binding assay on CHO-hY_2R cells. [b] Displacement of Dy-635-pNPY in a flow cytometric competition binding assay on CHO-hY_2R cells. (mean values, n = 2-4)

6.2 Chemistry

The majority of the highly selective potential radioligands exhibit lower Y_2R binding affinities than the parent compound BIIE 0246 (cf. Table 6.1 for a selection of potential [^{3}H]-radioligands; cf. Table 3.2 and Table 3.4 for binding constants and selectivity). Especially the carbamoyl-substituted analogs lose affinity compared to the corresponding amine precursors. However, several derivatives turned out to be highly potent Y_2R antagonists with affinities in the one-digit nM-range ((*S*)-**3.63**, (*S*)-**3.65**, (*S*)-**3.71**, **3.86**). Direct propionylation in N^G-position yielded **3.86**, the derivative with the highest Y_2R antagonistic activity.



Scheme 6.1. Preparation of the tritiated Y_2 receptor antagonist **6.1**. Reagents and conditions: a) (*S*)-**3.48** (40 eq), succinimidyl [^{3}H]-propionate (1 eq), NEt_3 (80 eq), CH_3CN, 20 h, rt.

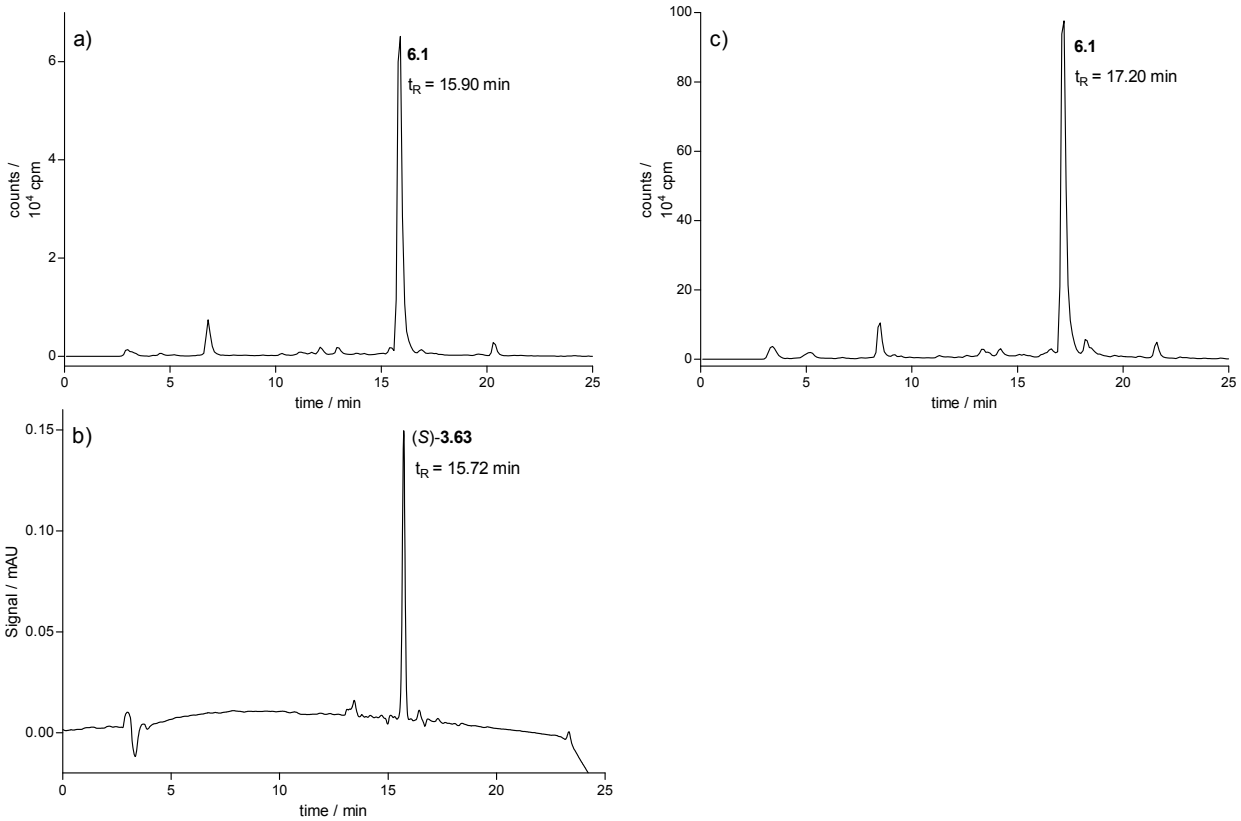


Figure 6.1. HPLC analysis of identity, purity and long-term stability (13 months) of [^{3}H]-UR-PLN196 (**6.1**). a) Radiochromatogram of the synthesized radioligand **6.1** (purity control). b) UV chromatogram of unlabeled ligand (*S*)-**3.63** (identity control). c) Long-term stability control of **6.1** with radiometric detection. Eluent: mixtures of CH_3CN + 0.05 % TFA (A) and 0.05 % aq. TFA (B), gradient: 0 to 20 min: A/B 30/70 to 60/40, 20 to 22 min: 60/40 to 95/5, 22 to 25 min: 95/5, columns: Agilent C18, 5 µm (chromatograms a) and b)), Synergi C-18, 5 µm (chromatogram c)). The minor difference in retention times (t_R) of a) and b) results from the setup of the UV and the radiodetector in series. The identity of **6.1** was confirmed by spiking with the "cold" analog (*S*)-**3.63**.

Considering Y_2R affinity, synthetic pathway, overall yield and supposed long-term stability, the amine precursor (*S*)-**3.48** was considered the most attractive candidate for labeling. The N^{ω}-[2,3-^{3}H]-propionyl-substituted argininamide **6.1** ([^{3}H]-UR-PLN196), the "hot" form of (*S*)-**3.63**, was prepared by acylation with the commercially available tritiated succinimidyl propionate (Scheme 6.1). After purification by HPLC, the designated radioligand **6.1** was obtained in a radiochemical purity of 90 % (Figure 6.1a; for comparison: (*S*)-**3.63** is obtained in 95 % chemical purity (Figure 6.1b)) with a specific activity of 73 Ci · mmol^{-1}. The identity of the radioligand was confirmed by HPLC analysis of labeled (**6.1**) and unlabeled UR-PLN196 ((*S*)-**3.63**), which have identical retention times (Figure 6.1a, b). Moreover, long-term stability in ethanol containing 100 µM TFA at –20 °C was proven over a period of 13 months (Figure 6.1c).

6.3 Pharmacological Results and Discussion

6.3.1 Saturation Binding

In the first binding experiments no saturation of the Y_2R with the newly developed radioligand **6.1** was observed, despite a binding affinity of the cold analog (*S*)-**3.63** in the one-digit nM range determined in flow cytometric competition binding experiments with various fluorescent ligands (cf. Figure 6.7b). Thus, the potential adsorption of the compound to different reaction vessels and in various solvents was investigated, as a high tendency towards the adsorption to plastics was already observed in case of BIIE 0246 in a previous work.[9] The results are presented in Figure 6.2.

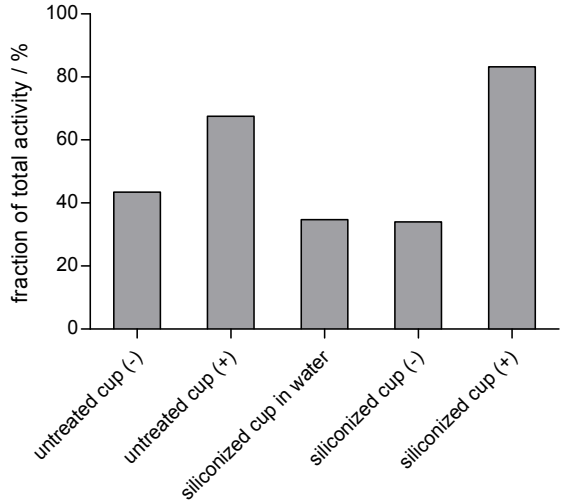


Figure 6.2. Fraction of total activity recovered after solution of **6.1** (c = 17 nM) in different solvents (water, binding buffer without BSA (–) and binding buffer with 1 % BSA (+)) to variously treated reaction vessels (cups). Siliconized cups were obtained by coating with Sigmacote^{TM} (Sigma-Aldrich, Germany). 100 % activity was calculated by means of the activity concentration a_V of the radioligand stock solution (a_V = 9.25 MBq · mL^{-1}; V (**6.1**) = 2 µL).

The highest fraction of recovered activity was obtained with siliconized reaction vessels in binding buffer + 1 % BSA (83 %). Adsorption increased when BSA was omitted. Thus, all dilutions were performed in siliconized cups and surface-modified

BD Primaria™ 24-well plates (Beckton Dickinson GmbH, Germany) were used to perform radioligand binding assays.

Saturation experiments with **6.1** at CHO-hY_2R cells afforded a K_D value of 65 nM, which is higher than expected from previous flow cytometric binding assays (K_i = 9.9 nM, cf. Table 6.1, cf. Figure 6.7b). Presumably, this discrepancy results from adsorption of small amounts of the radioligand to the surface-modified plates. Furthermore, the radioligand **6.1** might occupy a second low-affinity binding site. This is rather speculative and more data points with a higher number of repetitions within high concentrations would be necessary in order to detect a second binding site. Unfortunately, the performance of such additional assays was impossible due to the limited amounts of available hot ligand. Scatchard analysis revealed linearity, which is consistent with mass action behavior at a single site (Figure 6.3b).[10] Moreover, the radioligand showed a high specific binding throughout proving the applicability as a selective radioligand in binding studies on the Y_2R (Figure 6.3a). Besides the equilibrium dissociation constant K_D, saturation analysis with **6.1** revealed a maximum number of binding sites (B_{max}) of about 175,000 sites per cell.

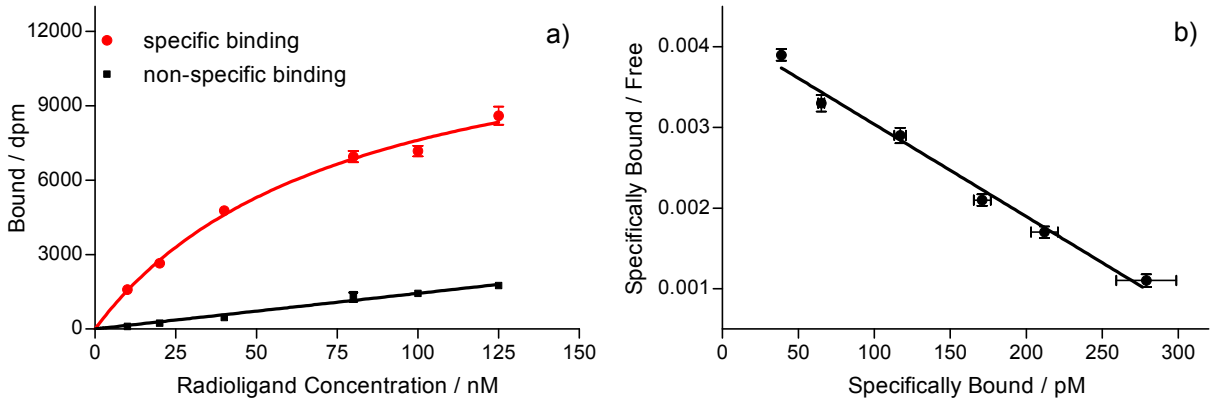


Figure 6.3. a) Representative saturation binding curve of **6.1** at CHO-hY_2R cells; b) Representative scatchard plot for the binding of **6.1** at CHO-hY_2R cells, best fitted by linear regression, K_D = –1/slope = 87 nM. (mean values ± SEM, n = 4, performed in triplicate)

6.3.2 Association and Dissociation Kinetics

The results from kinetic studies of **6.1** are presented in Figure 6.4. The novel Y_2R selective radioligand exhibits rather low on- and off-rates, which was also observed for fluorescence-labeled Y_2R antagonists (cf. Chapter 5). Association was mostly completed after 30 minutes (Figure 6.4a). Pseudo-irreversible binding was observed as a consequence of slow dissociation kinetics (≈ 20 % specifically bound radioligand after 90 minutes; Figure 6.4b). There are several examples of pseudo-irreversible binding, originating from slow dissociation even for lower molecular weight GPCR radioligands, *inter alia* the Histamine H_2 receptor agonist [^{3}H]-UR-DE257[11], or the NPY Y_5 receptor (Y_5R) selective antagonist [^{35}S]SCH 500946[12].

However, the equilibrium dissociation constant of **6.1**, calculated from the linearization of the kinetics (K_D = k_{off}/k_{on} = 43 nM; Figure 6.4c and d), was in good agreement with the K_D value derived from saturation binding experiments (K_D = 65 nM) proving that the radioligand **6.1** follows the law of mass action.[10]

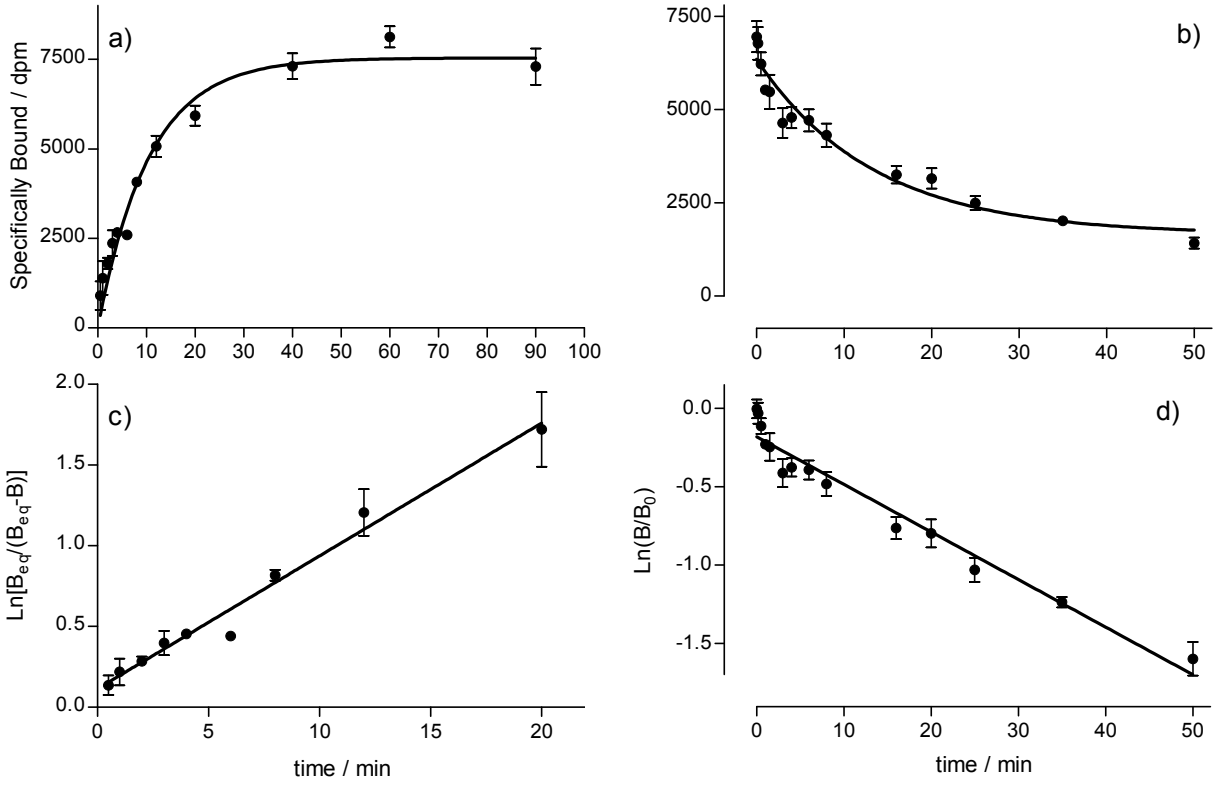


Figure 6.4. Association and dissociation kinetics of the specific Y_2R binding of **6.1** at CHO-hY_2R cells. a) Radioligand (c = 75 nM) association as a function of time; b) Radioligand (pre-incubation = 75 nM, 30 min) dissociation as a function of time, monophasic exponential decay, $t_{1/2}$ = 9.3 min, dissociation performed with 100-fold excess of BIIE 0246; c) Linearization $\ln[B_{eq}/(B_{eq}-B)]$ versus time of the association kinetic for the determination of k_{ob} and k_{on}, slope = k_{ob} = 0.082 min^{-1}, k_{on} = $(k_{ob} - k_{off})/[L]$ = 6.92 · 10^{-4} min^{-1} · nM^{-1}; d) Linearization $\ln(B/B_0)$ versus time of the dissociation kinetic for the determination of k_{off} = slope · (–1) = 0.030 min^{-1}. (mean values ± SEM, n = 3)

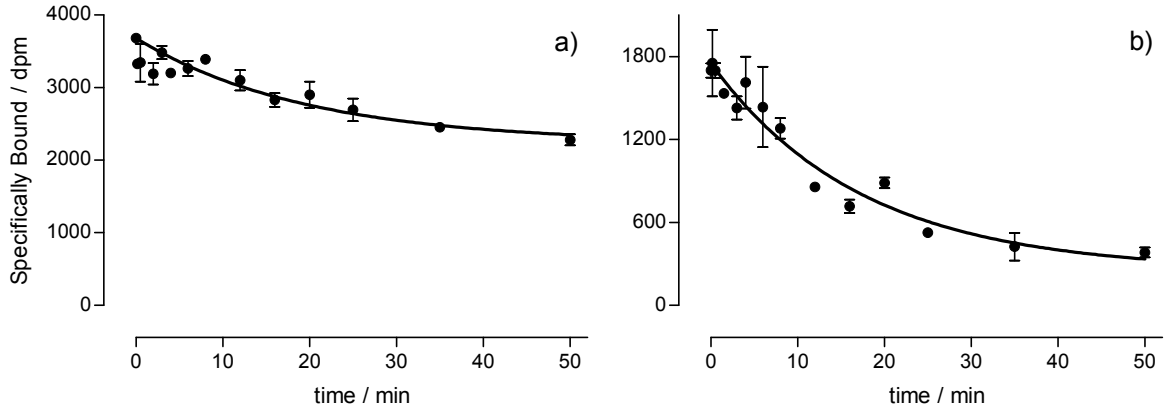


Figure 6.5. Radioligand dissociation kinetics of **6.1** on CHO-hY_2R cells. a) Dissociation in the presence of 100-fold excess of the endogenous agonist pNPY. b) Dissociation in the presence of 400-fold excess of the Y_2R antagonistic small molecule SF-11[13]. (mean values ± SEM, n = 3)

Dissociation experiments were also performed in the presence of pNPY or the low molecular weight Y_2R antagonist SF-11[13] instead of BIIE 0246, the parent compound of **6.1**. Displacement with structurally distinct compounds should give insights into the binding behavior of the novel radioligand. As already observed for the novel fluorescent ligands no dissociation of the argininamide-type ^{3}H-labeled antagonist was observed with pNPY (Figure 6.5a), whereas the dissociation induced by the non-peptide antagonist SF-11 was the same as in the presence of BIIE 0246 (Figure 6.5b). Hence, **6.1** is displaceable by non-peptide antagonists such as SF-11 or BIIE 0246, but not by the peptide pNPY.

6.3.3 Y_2R Antagonism of 6.1: Ca^{2+} Assay, Schild Analysis and Displacement with pNPY

The interpretation of agonist concentration-response curves (CRCs) in the presence of an antagonist is indispensable for the elucidation of the antagonist´s binding mode. As defined by Vauquelin *et al*. insurmountable antagonism refers to experiments in which the cells are pre-incubated with the antagonist before the addition of the agonist and the measurement of the response. Yet, non-competitive antagonism can only be positively identified by co-incubation experiments.[14] Thus, the Y_2R antagonism of (*S*)-**3.63** was investigated in a fura-2 based Ca^{2+} assay on CHO-hY_2R cells for co-application and after pre-incubation for 20 minutes, respectively (Figure 6.6a and b). CRCs were constructed in the absence and presence of the antagonist at different concentrations and the data were subjected to Schild analysis[15] (Figure 6.6c).

On the one hand, co-application with increasing concentrations of the “cold” ligand (*S*)-**3.63** led to a parallel rightward shift of the CRCs of pNPY indicating competitive antagonism as already observed for BIIE 0246 in similar assays[16-17]. Unfortunately, pNPY concentrations >3 µM result in a depression of the Ca^{2+}-response. Hence, the maximal effect was not detectable for CRCs generated in the presence of high antagonist concentrations (Figure 6.6a). On the other hand, pretreatment of the cells with (*S*)-**3.63** for 20 minutes reduced the maximal agonist response strongly in a concentration-dependent manner with comparatively low effects on the EC_{50} values of pNPY (Figure 6.6b). The strong depression of the maxima after pre-incubation indicates an insurmountable antagonism against pNPY.[14, 17] Schild regression, constructed with the CRCs for co-application with (*S*)-**3.63** (Figure 6.5a), yielded a linear plot with a slope steeper but not significantly different from unity (Figure 6.5c). The pA_2 value reflects the affinity of the antagonist and the resulting A_2 value (43 nM) equals the K_D value determined from the study of binding kinetics.

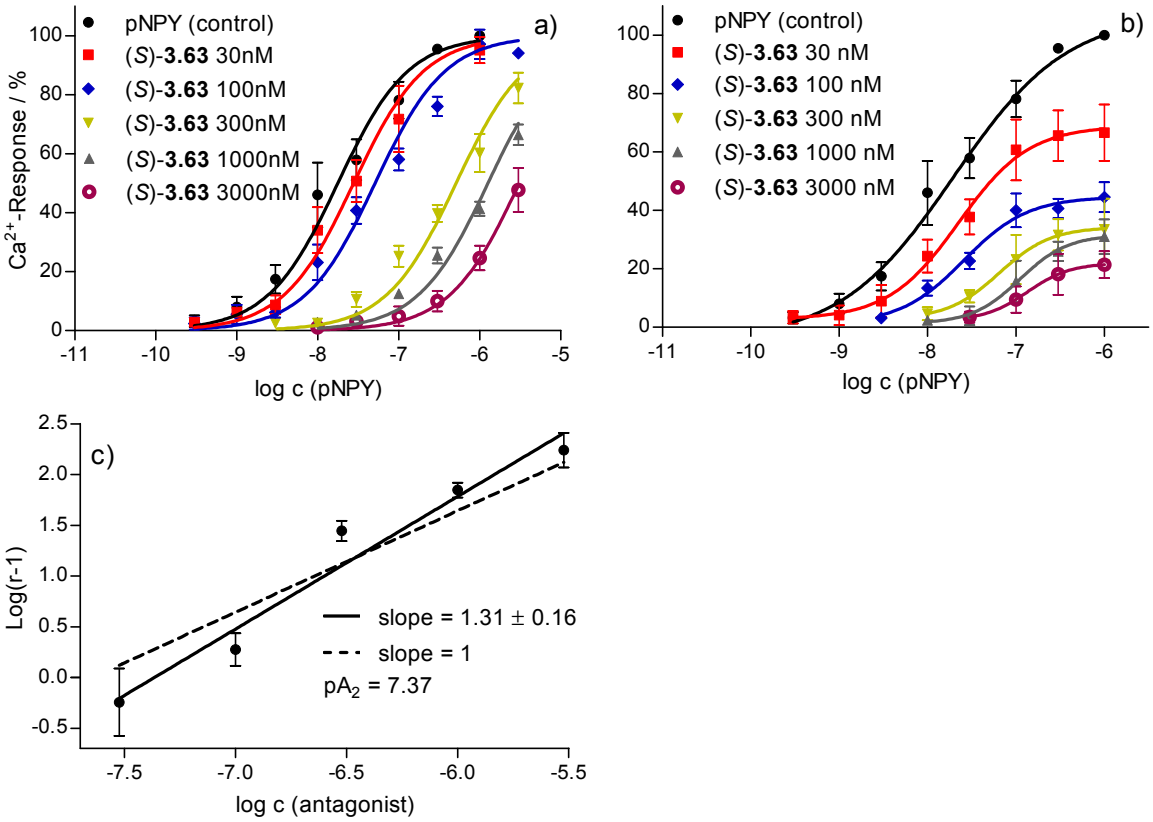


Figure 6.6. Concentration-response curves (CRCs) of pNPY obtained from a fura-2 assay on CHO-hY_2R cells and Schild analysis. The presence of (*S*)-**3.63** (cold form of **6.1**) led to a rightward shift of the curves. a) Co-incubation with different concentrations of antagonist (*S*)-**3.63**. b) Pretreatment with different concentrations of antagonist (*S*)-**3.63** for 20 minutes. c) Schild regression: log(r–1) plotted against log antagonist concentration; the concentration ratios r (r = $10^{\Delta pEC50}$) were calculated from the rightward shifts (ΔpEC_{50}) of the CRCs when co-incubated with (*S*)-**3.63** as shown in Figure 6.6a. pA_2 = 7.37; A_2 = 43 nM. (mean values ± SEM, n = 3-8)

Recently, a Y_5R selective radioligand with similar properties in kinetic and functional experiments was reported as an insurmountable pseudo-irreversible non-peptide antagonist.[12] There are several possible explanations for an apparently insurmountable pseudo-irreversible binding. A slow rate of receptor dissociation[18], a slow rate of interconversion from an inactive to an active receptor conformation[19-20] and a stabilization of an inactive ligand (antagonist) specific receptor conformation[21], respectively, or binding to a site distinct from the peptide agonist binding site[22]. Interestingly, pNPY displaced the radioligand **6.1** in competition binding only at very high concentrations (Figure 6.7a; K_i = 406 ± 113 nM), whereas in binding experiments with [^{3}H]-pNPY the endogenous peptide showed a very high affinity with a 1000-fold lower dissociation constant (K_i = 0.4 ± 0.1 nM)[23]. Additionally, pNPY was not able to fully displace **6.1** in dissociation experiments (Figure 6.5a). These data suggest that BIIE 0246 derived antagonists bind to a different site on the Y_2R than the endogenous peptide NPY, or at least, the binding sites overlap only partially. Apparently, argininamide-type Y_2R antagonists are characterized by two features: firstly, slow dissociation from the receptor and secondly, occupation of a distinct binding site and – possibly – the stabilization of a ligand-specific conformation.

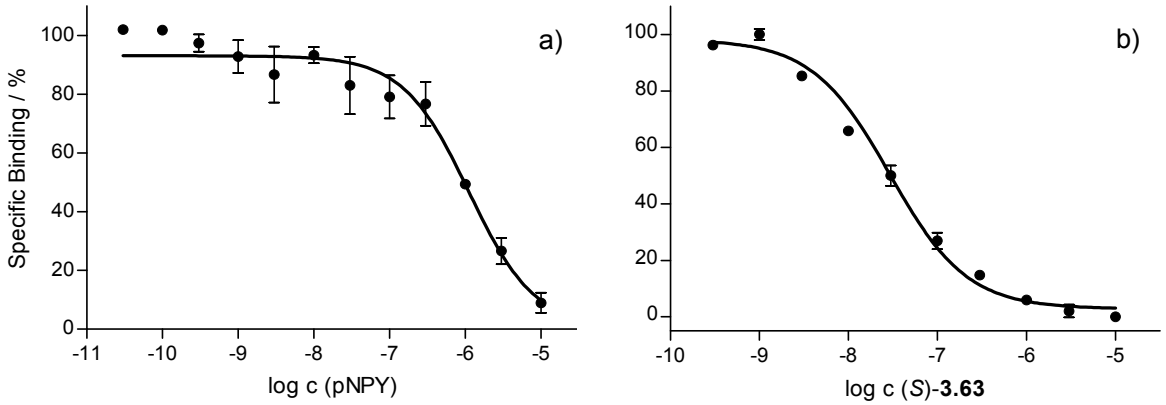


Figure 6.7. a) Displacement of the tritiated Y_2R antagonist **6.1** (c = 75 nM) by the agonist pNPY. b) Flow cytometric competition binding assay. Displacement of Dy-635-pNPY (c = 5 nM) by the "cold" radioligand (*S*)-**3.63**. The assays were performed on CHO cells stably expressing the Y_2R with an incubation period of 120 min. (mean values ± SEM, n = 3)

Table 6.2. Y_2R binding and functional characteristics of (*S*)-**3.63**/**6.1**.

k_{off} / min^{-1} [a]	k_{on} / $min^{-1} \cdot nM^{-1}$ [b]	k_{off}/k_{on} / nM [c]	K_D / nM [d]	A_2 / nM [e]
0.030	0.000692	43	65	43

[a] Dissociation rate constant from linear regression. [b] Association rate constant from linear regression. [c] Kinetically derived dissociation constant. [d] Equilibrium dissociation constant determined in saturation binding experiments. [e] Antagonist dissociation constant of (*S*)-**3.63** derived from Schild analysis.

6.3.4 Competition Binding Experiments: Application as Standard Ligand

Although being insurmountable against pNPY, the radioligand **6.1** was successfully applied as standard ligand in competition binding studies of non-peptide Y_2R antagonists as shown in Figure 6.8. As the receptor was not completely saturable, the K_D value received from binding kinetics was applied for the calculation of K_i values by means of the Cheng-Prusoff equation[24]. The K_i value of BIIE 0246 (K_i = 13 nM) is consistent with reported data from competition binding using radio-labeled agonists (K_i = 24 nM[9] and 36 nM[16], resp.). Furthermore, the dissociation constants of the other investigated compounds are in good agreement with data from flow cytometry (Table 6.3). However, structurally diverse and selective Y_2R antagonists as reference compounds would be necessary for the verification of these results. In agreement with theory, the K_i value of (*S*)-**3.63** from competition binding experiments with the labeled analog **6.1** corresponds, within the error range of the methods, to the K_D value of compound **6.1**: 36 nM for (*S*)-**3.63** and 43 nM for **6.1**.

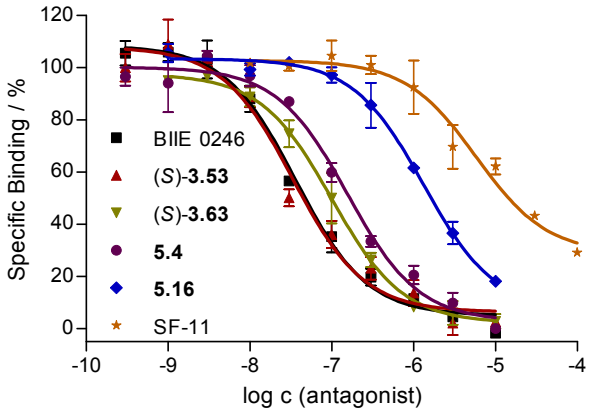


Figure 6.8. Displacement of the [^{3}H]-labeled radioligand **6.1** (c = 75 nM, K_D = 43 nM) by arginin-amide-type Y_2R antagonists BIIE 0246, (*S*)-**3.53**, (*S*)-**3.63** (cold form of **6.1**), fluorescent Y_2R antagonists **5.4** and **5.16** and the low molecular weight Y_2R selective antagonist SF-11. The assay was performed on CHO cells stably expressing the Y_2R with an incubation period of 30 min. (mean values ± SEM, n = 2-3)

Table 6.3. K_i values from radioligand competition binding experiments using **6.1** as radioligand compared to data from flow cytometric competition binding assays with Cy5-pNPY and **5.4**, respectively.

Compd.	Radioligand binding, K_i / nM displacement of **6.1**[a]	Flow cytometry, K_i / nM displacement of Cy5-pNPY[b]	Flow cytometry, K_i / nM displacement of **5.4**[c]
BIIE 0246	13 ± 1	10 ± 1	22 ± 6
(*S*)-**3.50**	28 ± 5	16 ± 3	--
(*S*)-**3.53**	12 ± 2	2.1 ± 0.3	--
(*S*)-**3.63**	36 ± 14	9.9 ± 1.1[d]	77 ± 3
3.86	33 ± 7	5.2 ± 1.8	--
5.4	57 ± 4	23 ± 4	--
5.16	482 ± 19	--	982 ± 207
SF-11	2118 ± 975	1251 ± 348	4915 ± 836

[a] K_D = 43 nM (kinetics), c = 75 nM. [b] K_D = 5.2 nM (Fl-4), c = 5 nM. [c] K_D = 25 nM (Fl-2), c = 40 nM. [d] Determined with Dy-635-pNPY. (mean values ± SEM, n = 2-5). All experiments performed at CHO-hY_2R cells.

6.4 Summary and Conclusion

The guanidine-acylguanidine bioisosteric approach was applied to the preparation of a tritiated Y_2R selective radioligand starting from the highly potent argininamide-type Y_2R antagonist BIIE 0246. Acyl and carbamoyl moieties, bearing a terminal primary amino group for radiolabeling, were attached at N^G of the guanidine function in BIIE 0246 and optimized with respect to length and chemical nature of the linker. Therefore, the "cold" versions of the corresponding propionamides were investigated in a flow cytometric Y_2R binding assay at CHO cells expressing the hY_2R to select the most suitable candidate for radiolabeling. A one step synthesis afforded the desired radioligand **6.1** by acylation of the amine precursor, the N^G-[5-(2-aminoethylamino)-5-oxopentanoyl]-substituted BIIE 0246 ((*S*)-**3.48**), with succinimidyl [2,3-^{3}H]-propionate (specific activity: 73 Ci · mmol^{-1}).

The non-peptidic radioligand [^{3}H]-UR-PLN196 is superior to Y_2R-addressing radiolabeled peptides due to selectivity, resistance against enzymatic cleavage, long-term stability, mode of action (antagonist vs. agonist) and costs of preparation. [^{3}H]-UR-PLN196 can be used for the detection and quantification of Y_2R binding sites. Detailed investigations in binding experiments (with **6.1**) and functional assays (with (*S*)-**3.63**) identified the novel radioligand as an insurmountable antagonist versus pNPY exhibiting pseudo-irreversible binding at the Y_2R. Moreover, the tritiated compound was successfully applied as standard ligand in competition binding experiments with several Y_2R antagonists.

In conclusion, the prepared radioligand **6.1** proved to be a valuable pharmacological tool for the detection of the Y_2R *in vitro* and for the investigation of the antagonistic binding mode, respectively, as well as for the characterization of non-peptidic Y_2 receptor antagonists.

6.5 Experimental Section

6.5.1 General Experimental Conditions

Chemicals and solvents were purchased from commercial suppliers and used without further purification. Porcine NPY (pNPY) was kindly provided by the Institute of Organic Chemistry I at the Ruhr-University Bochum (Prof. Dr. C. Cabrele). SF-11 was purchased from Tocris Bioscience (Bristol, United Kingdom). All solvents were of analytical grade or distilled prior to use.

Analytical HPLC analysis and purification, respectively, were performed on a Waters system (two pumps 510, pump control module, 486 UV detector, Packard Radiomatic Flow-1 beta Series A-500 detector, flow rate of liquid scintillator (Rotiszint^{TM} eco plus, Carl Roth GmbH & Co. KG, Karlsruhe, Germany): 4 mL/min). In general, an Agilent Scalar C18 column (250 × 4.6 mm, 5 µm) was used. Long-term stability control was performed on a Synergi C-18 column (250 × 4.6 mm, 5 µm).

6.5.2 Synthesis, Purification and Quantification of [^{3}H]-UR-PLN196 (6.1)

Succinimidyl [2,3-^{3}H]-propionate was purchased from Hartmann Analytic GmbH (Braunschweig, Germany) and provided in hexane/ethyl acetate 9:1 (v/v) (specific activity a_s = 3.07 TBq mmol^{-1} = 83 Ci mmol^{-1}; 185 MBq/2.5 mL).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]-*N*$^{\omega}$-[4-(2-[2,3-^{3}H]propanoylaminoethyl)aminocarbonylbutanoyl]arginin-amide (6.1). Compound (*S*)-**3.48** (1.23 mg, 0.964 µmol) was dissolved in anhydrous CH_3CN (140 µL) yielding a concentration of 6.9 mM, and NEt_3 was diluted in CH_3CN to a concentration of 2.7 µL NEt_3/100 µL. Compound (*S*)-**3.48** (6.9 mM in CH_3CN, 50 µL, 0.344 µmol) and NEt_3 (10 µL of the above described solution in CH_3CN, 1.928 µmol) were added to succinimidyl [2,3-^{3}H]-propionate (4.22 µg, 0.0241 µmol) in 1 mL hexane/ethyl acetate 9:1 (v/v) in a 1.5 mL Eppendorf reaction vessel (screw top). The solvent was removed in a vacuum concentrator (40 °C) over a period of 20 min and (*S*)-**3.48** (6.9 mM in CH_3CN, 90 µL, 0.620 µmol) was added. Again, the solvent was removed in a vacuum concentrator (40 °C) over a period of 40 min. After addition of 100 µL of CH_3CN the reaction mixture was vigorously blended (vortexer) for 1 min, briefly centrifuged and stirred with a magnetic micro stirrer at rt overnight.
For HPLC analysis 0.5 µL of the reaction mixture (reaction control) were diluted (1:200) with 97.5 µL of CH_3CN/0.05 % aq. TFA (30/70) and spiked with 2 µL of "cold" radioligand (*S*)-**3.63** (1 mM in CH_3CN) to a total volume of 100 µL. This solution was completely injected into the HPLC system and analyzed by means of UV- and radiometric detection (Figure 6.9b). Eluent: CH_3CN + 0.05 % aq. TFA (A) and 0.05 % aq. TFA (B), gradient: 0 to 25 min: A/B 30/70 to 55/45, 25 to 27 min: 55/45 to 95/5, 27 to 35 min: 95/5. Afterwards, the reaction was stopped with 10 % aq. TFA (2.9 µL, corresponds to ≈ 160 eq of TFA) and the reaction mixture was purified with analytical HPLC (Figure 6.9a). Therefore, the reaction mixture was diluted with 0.1 % aq. TFA (350 µL) and the product was isolated (3 injections) using the conditions specified for the reaction control. For this purpose radiometric detection was not performed. The product was eluted at 18.8 to 19.3 min and collected in a 1.5 mL Eppendorf reaction vessel (screw top), which was put into the vacuum concentrator between the injections. The combined product fractions were evaporated to dryness in a vacuum concentrator, the residue was dissolved in 300 µL of ethanol containing TFA (100 µM) and transferred to a clean 3 mL Amersham glass vial together with the washings (2 × 100 µL).

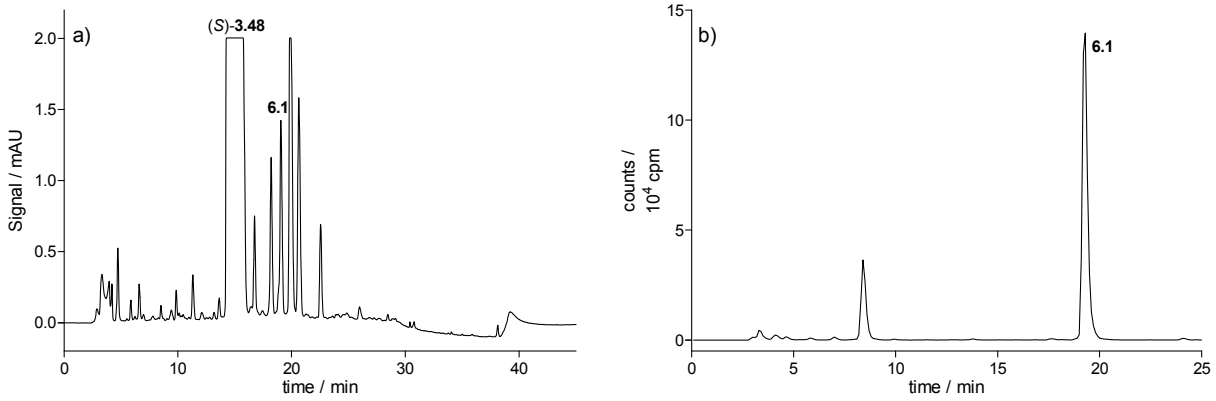


Figure 6.9. a) Analytical HPLC run for the isolation of radioligand **6.1**; detection: UV (220 nm). b) HPLC analysis of the reaction mixture containing the non-purified radioligand **6.1**; radiometric detection, t_R = 19.30 min.

Quantification: A five point calibration was performed with (*S*)-**3.63** (0.25, 0.5, 1.0, 1.5 and 3.0 µM, injection volume: 100 µL; Figure 6.10). Eluent: CH_3CN + 0.05 % aq. TFA (A) and 0.05 % aq. TFA (B), gradient: 0 to 20 min: A/B 30/70 to 60/40, 20 to 20 min: 60/40 to 95/5, 22 to 30 min: 95/5 (retention time: 15.72 min). The solutions for injection were prepared in CH_3CN/0.05 % aq. TFA (20/80) less than 5 minutes prior to the injection. All standard solutions were prepared from a 40 µM solution of (*S*)-**3.63** (in CH_3CN/0.05 % aq. TFA 20/80), which was freshly made from a 1 mM stock solution of (*S*)-**3.63** in CH_3CN. Two aliquots (2.0 µL) of the ethanolic solution (total volume: 500 µL) of the product were diluted with 100 µL of CH_3CN/0.05 % aq. TFA (20/80), and 100 µL were analyzed by HPLC. Whereas one sample was only used for quantification of the product by UV detection the second sample was additionally monitored radiometrically to determine radiochemical purity (Figure 6.1a and b). The molarity of the ethanolic solution of **6.1** was calculated from the mean of the peak areas and the linear calibration curve obtained from the peak areas of the standards. Yield: 9.1 µg (7.41 nmol, 31 %).

Determination of the specific activity: 1.5 µL of the ethanolic solution were diluted with 448.5 µL of a mixture of CH_3CN and water (50/50) in duplicate, and 9 µL of the 1:300 dilutions were counted in Rotiszint^{TM} eco plus (3 mL). Calculated specific activity (a_s): 2.71 TBq · mmol^{-1} (73.3 Ci · mmol^{-1}). The activity concentration (a_v) was adjusted to 9.25 MBq · mL^{-1} (0.25 mCi · mL^{-1}) by addition of ethanol (1685 µL) containing TFA (100 µM) to the residual solution yielding a molarity of 3.42 µM. The radioligand **6.1** was stored at –20 °C.

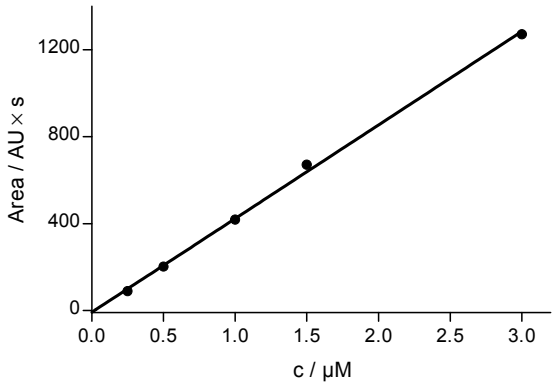


Figure 6.10. Calibration line for the quantification of the molarity of the ethanolic solution of **6.1**. Five point calibration was performed with (*S*)-**3.63** (unlabeled analog of **6.1**). Peak areas calculated from UV detection. Straight line equation: $y = 430.963\ AU \cdot s \cdot \mu M^{-1} \cdot x - 8.159\ AU \cdot s$. Goodness of fit $r^2 = 0.9985$.

6.5.3 Pharmacology: Experimental Protocols

General. The buffer for the Y_2R binding studies of **6.1** was prepared by the addition of 1 % BSA to a buffer (pH 7.4) consisting of HEPES (25 mM), $CaCl_2 \times 2\ H_2O$ (2.5 mM) and $MgCl_2 \times 6\ H_2O$ (1 mM). Bacitracin (100 µg/mL) was added for binding studies with pNPY. This HEPES buffer without the additives was used to wash the cells previous to and after radioligand binding. The loading buffer (pH 7.4) for the determination of intracellular Ca^{2+} mobilization in CHO cells was prepared by dissolving NaCl (120 mM), KCl (5 mM), $MgCl_2 \times 6\ H_2O$ (2 mM), $CaCl_2 \times 2\ H_2O$ (1.5 mM), HEPES (25 mM) and glucose (10 mM). The lysis solution for the radioligand binding assays consisted of urea (8 M), acetic acid (3 M), and Triton-X-100 (1 %) in water. Stock solutions of all compounds were prepared in DMSO (2 or 10 mM) and stored at –20 °C. Tritium counting was done in 3 mL of Rotiszint™ eco plus with a Beckman LS 6500 beta-counter.

Cell culture. CHO cells were cultured as described elsewhere.[23]

Radioligand binding assay. The radioligand binding assay was performed as essentially described.[23] Cells were seeded in 24-well plates 2-3 days prior to the experiment. On the day of the experiment confluency of the cells was at least 70 %. The culture medium was removed by suction, the cells were washed with once with buffer (500 µL), and covered with binding buffer (200 µL). Binding buffer (25 µL) and binding buffer (25 µL) with **6.1** (10-fold concentrated) was added for total binding. For non-specific binding and displacement of **6.1** binding buffer (25 µL) with the competing agent (10-fold concentrated) and binding buffer (25 µL) with **6.1** (10-fold concentrated) was added. During incubation at room temperature the plates were gently shaken. After incubation (saturation experiments and competition with BIIE 0246, SF-11, (*S*)-**3.50**, (*S*)-**3.53**, (*S*)-**3.63**, **3.86**, **5.4**, **5.16**: 30 min; competition with pNPY: 2 h) the binding buffer was removed, the cells were washed twice with buffer (500 µL, 4 °C, ≈ 30 s), covered with lysis solution (200 µL) and the plates were gently

shaken for at least 30 min. The lysates were transferred into scintillation vials filled with scintillator (3 mL) and the dishes were washed once with lysis solution (100 µL). In case of the investigation of dissociation kinetics the cells were incubated with binding buffer (250 µL) containing the competing agent (BIIE 0246, SF-11: 100-fold higher concentrated than the previously used radioligand; pNPY: 400-fold higher concentrated than the previously used radioligand) after incubation with radioligand **6.1** (75 nM) and subsequent washing (twice 500 µL, 4 °C). After different periods of time the cells were washed again twice with buffer (500 µL, 4 °C) followed by the addition of lysis solution. For association kinetics cells were incubated with **6.1** (75 nM) in binding buffer (250 µL). The supernatant was sucked off after different periods of time and the adherent cells were washed twice with cold buffer (500 µL, 4 °C) before lysis solution was added. The non-specific binding was determined with BIIE 0246 (100 fold higher concentrated than the previously used radioligand) in kinetics. Assays were performed in triplicate.

Fura-2 assay on CHO-cells (Schild analysis). The fura-2 assay was performed as previously described[25] using a LS50 B spectrofluorimeter from Perkin Elmer (Überlingen, Germany).
Concentration-response curves of pNPY were constructed in the presence of (*S*)-**3.63** at different concentrations. The signal intensities were related to the maximal intensity obtained from a pNPY effect curve in the absence of (*S*)-**3.63**. For Schild analysis, $\log_{10}$ (r-1) was plotted against $\log_{10}$ c (antagonist) with $r = 10^{\Delta pEC50}$.
ΔpEC_{50}: rightward shift of the concentration-response curves in the presence of different concentrations of antagonist.

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Chapter 7 Summary

Neuropeptide Y (NPY), a 36 amino acid peptide, is widely distributed in the central and peripheral nervous system, where it acts as a neurotransmitter and exhibits a large number of physiological functions, including the regulation of blood pressure, control of food intake, anxiety, pain and hormone secretion. In humans, these effects are mediated by four receptor subtypes (Y_1, Y_2, Y_4, Y_5), all belonging to class A of the superfamily of G-protein coupled receptors (GPCRs). The Y_2R is expressed, e. g., in sympathetic nerve endings, in the renal tubules and in distinct brain regions such as the hippocampus and the hypothalamus. It is discussed to be involved in several human diseases, for instance, epilepsy, obesity and cancer, and therefore regarded as an attractive target in drug design. However, antagonistic pharmacological tools for the elucidation of the receptor´s function in health and disease are still missing.

In 1999, the (*S*)-argininamide BIIE 0246, a C-terminal mimic of NPY, was reported the first highly potent and selective Y_2R antagonist. N^G-Acylation of BIIE 0246 was demonstrated to retain or even increase the Y_2R affinity, thereby improving the pharmacokinetic properties. Thus, we prepared N^ω-aminoacylated analogs as precursors towards radio- and fluorescence labeled as well as bivalent pharmacological tools according to the guanidine-acylguanidine bioisosteric approach. Such derivatives, bearing a free amino group, exhibited the highest Y_2R affinities (K_i < 10 nM). Interestingly, masking the positive charge, e.g. by acylation, resulted in decreased affinities, presumably, due to the loss of an additional electrostatic interaction of the primary amine with the receptor.

Bivalent ligands were synthesized starting from various amine precursors derived from BIIE 0246 by acylation with different aliphatic dicarboxylic acids. Only minor differences in affinities (K_i = 61-300 nM) were observed for the majority of the compounds, irrespective of the diversity in length and chemical nature of the spacer. However, bivalent ligand **4.6**, constructed from an amine precursor bearing an additional amino group in the linker, showed a binding affinity (K_i = 21 nM) and antagonistic activity (K_B = 15 nM) in the same range as the monovalent antagonists, corroborating the affinity-enhancing effect of a positive charge in the linker.

Fluorescence ligands were synthesized from the amine precursors by acylation with succinimidyl esters or by ring transformation of pyrylium dyes. In terms of retaining affinity, the pyrylium dyes were superior to the bulky cyanine, hemicyanine and Bodipy dyes. Whereas the majority of the cyanine and hemicyanine labeled fluorescent ligands proved to be suitable for the detection of Y_2Rs at the cell membrane by confocal microscopy, rapid cellular uptake was observed for most of the Py-1 and Py-5 coupled antagonists. Based on the results from binding studies and confocal microscopy, four fluorescent Y_2R antagonists (**5.4**, **5.15**-**5.17**) were chosen for flow cytometric binding studies. These compounds turned out to be applicable as labeled standard ligands in saturation and competition binding experiments. Interestingly, kinetic studies revealed a pseudo-irreversible binding of **5.16** at the Y_2R. The endogenous ligand NPY was not able to displace the novel red-fluorescent Y_2R antagonists **5.4** and **5.16** even at very high concentrations.

Acylation of the amine precursor (*S*)-**3.48** with succinimidyl [2,3-^{3}H]-propionate afforded the easily accessible, highly potent and selective tritiated Y_2R antagonist [^{3}H]-UR-PLN196 (A_2: 43 nM, Ca^{2+} assay; K_D: 43 nM, determined by kinetic studies), which was shown to be useful for the quantification of Y_2R binding sites and for the radiochemical determination of Y_2R binding affinities of small molecules. In addition, the novel radioligand was identified as an insurmountable antagonist against pNPY exhibiting pseudo-irreversible binding at the Y_2R similar to the fluorescent labeled antagonist **5.16**.

In conclusion, this work presents a straight-forward synthetic route towards labeled argininamide-type Y_2R antagonists related to BIIE 0246 with improved physico-chemical properties. These compounds are useful pharmacological tools for the detection of the Y_2R *in vitro* and for detailed investigations of the antagonistic binding mode, respectively, as well as for the identification and characterization of small molecule Y_2R antagonists. Moreover, these tracers are suitable to replace labeled NPY standard ligands in binding assays due to their beneficial properties in terms of Y_2 receptor subtype selectivity, antagonistic mode of action, stability and kinetics.

Chapter 8 Appendix

8.1 Abbreviations

aq.	aqueous
Ar	aryl
a_s	specific activity
a_v	activity concentration
BBB	blood brain barrier
B_{max}	maximum number of binding sites
BIIE 0246	(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazine-1-yl]ethyl}cyclopentyl)acetyl]argininamide
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bq	becquerel
br	broad singulet
BSA	bovine serum albumin
^{t}Bu	*tert*-butyl
Bz	benzoyl
c	concentration
$[Ca^{2+}]_i$	intracellular calcium ion concentration
Cbz	benzyloxycarbonyl
CD	circular dichroism
CHO	chinese hamster ovary
Ci	curie
CI	chemical ionization
CLSM	confocal laser scanning microscopy
CNS	central nervous system
COSY	correlated spectroscopy
CRC	concentration-response curve
d	doublet
δ	chemical shift
DCC	*N*,*N´*-dicyclohexylcarbodiimide
DCU	*N*,*N´*-dicyclohexylurea
DIPEA	*N*,*N´*-diisopropyl-ethylamine
DMAP	4-(dimethylamino)pyridin
DMF	dimethylformamide

DMSO	dimethylsulfoxide
DMSO-d_6	per-deuterated dimethylsulfoxide
EC_{50}	agonist concentration which induces 50 % of maximum response
EDC	*N*-(3-dimethylaminopropyl)-*N´*-ethyldicarbodiimide
EI	electron impact ionization
eq	equivalent(s)
EtOH	ethanol
FAB	fast atom bombardment
FACS	fluorescence activated cell sorter
FCS	fetal calf serum
Fmoc	9-fluorenylmethoxycarbonyl
FRET	fluorescence-resonance energy transfer
GPCR	G-protein coupled receptor
h	hour(s)/human
HEC-1B	human endometrial carcinoma
HEL	human erythroleukemia
HEPES	2-(4-(2-hydroxyethyl)-1-piperazinyl)ethansulfonic acid
HMBC	heteronuclear multiple bond correlation
HOBt	1-hydroxybenzotriazole
HPLC	high-pressure liquid chromatography
HR-MS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
HTS	high throughput screening
IBCF	isobutyl chloroformate
IC_{50}	antagonist concentration which suppresses 50 % of an agonist induced effect or displaces 50 % of a labeled ligand from the binding site
IR	infrared
^nJ	coupling constant
k	retention (capacity) factor
K_B	dissociation constant derived from a functional assay
K_D	dissociation constant derived from a saturation experiment or kinetics
K_i	dissociation constant derived from a competition binding assay
k_{ob}	observed/macroscopic association rate constant
k_{off}	dissociation rate constant
k_{on}	association rate constant
LSI-MS	liquid secondary ion mass spectrometry
m	multiplet
M	molar (mol/L)
MeOH	methanol
mp	melting point
MS	mass spectrometry

MW	microwave
NHS	*N*-hydroxysuccinimide
NMR	nuclear magnetic resonance
NPY	neuropeptide Y
p	quintet
PBS	phosphate buffered saline
PET	positron emission tomography
Ph	phenyl
Pht	phthaloyl
ppm	parts per million
quat	quartet
QY	quantum yield
RP	reversed phase
rt	room temperature
s	singulet/second(s)
SAR	structure-activity relationships
SEM	standard error of the mean
t	triplet/time
t_0	hold-up time
t_R	retention time
TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TM	transmembrane domain
TMSI	trimethylsilyl iodide
TMSOTf	trimethylsilyl triflate
UV	ultraviolet
Y_n	NPY receptor subtypes, n = 1, 2, 4, 5, 6

8.2 Flow Cytometric Saturation and Competition Binding Experiments

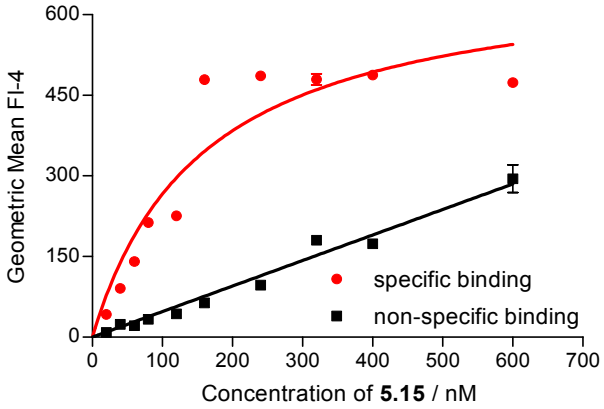

Figure 8.1. Flow cytometric saturation binding experiment with fluorescent ligand **5.15** on CHO-hY_2R cells (mean values ± SEM, n = 2, experiments performed in triplicates). Non-specific binding was determined in the presence of BIIE 0246 (100-fold excess); incubation time: 30 min.

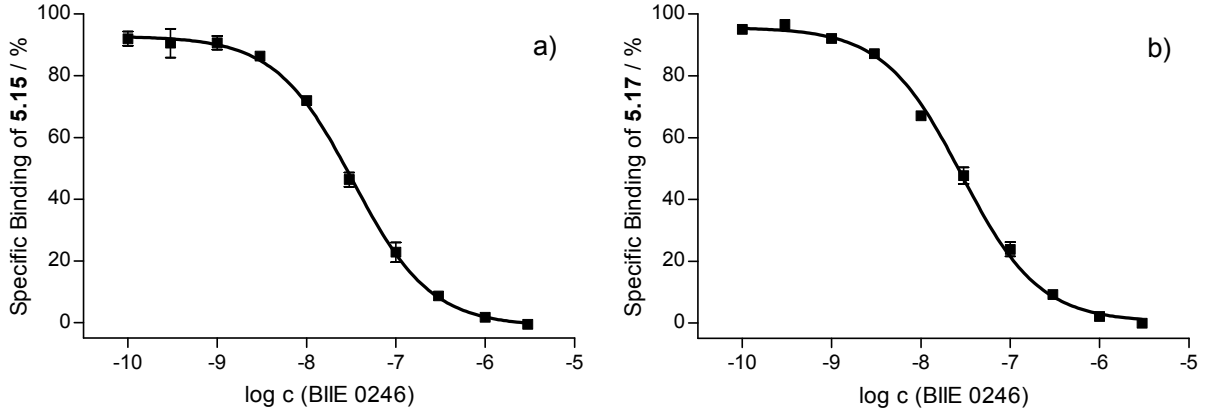

Figure 8.2. Flow cytometric competition binding on CHO-hY_2R cells; incubation time: 30 min; non-specific binding was determined with BIIE 0246 (c = 10 µM); mean values ± SEM (n = 2); measured in FI-4. a) Displacement of **5.15** (c = 100 nM, K_D = 143 nM). b) Displacement of **5.17** (c = 40 nM, K_D = 42 nM).

8.3 Confocal Microscopy

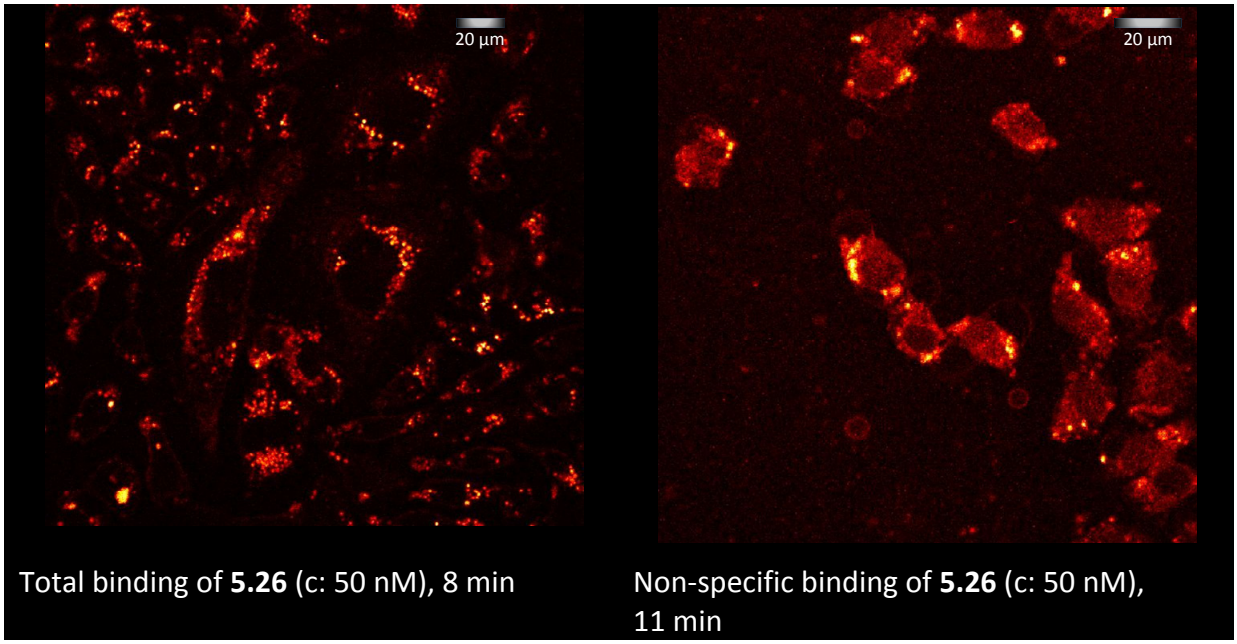

Figure 8.3. Binding of the fluorescent ligand **5.26** to Y_2R expressed in the cell membrane of CHO-hY_2R cells, visualized by confocal microscopy. Non-specific binding determined in the presence of BIIE 0246 at 100-fold excess. Cells were incubated with the fluorescent ligands at rt in Leibowitz L15 culture medium. All images were acquired with a Zeiss Axiovert 200 M microscope.

8.4 HPLC Stability Investigations

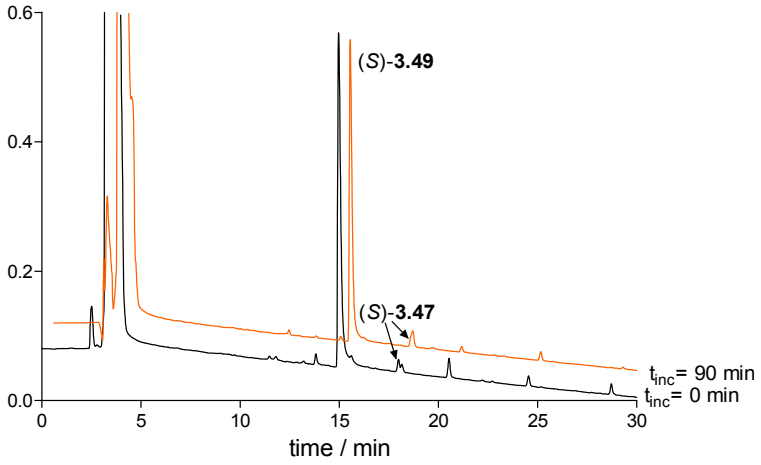

Figure 8.4. HPLC analysis of the Y_2R antagonist (*S*)-**3.49** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

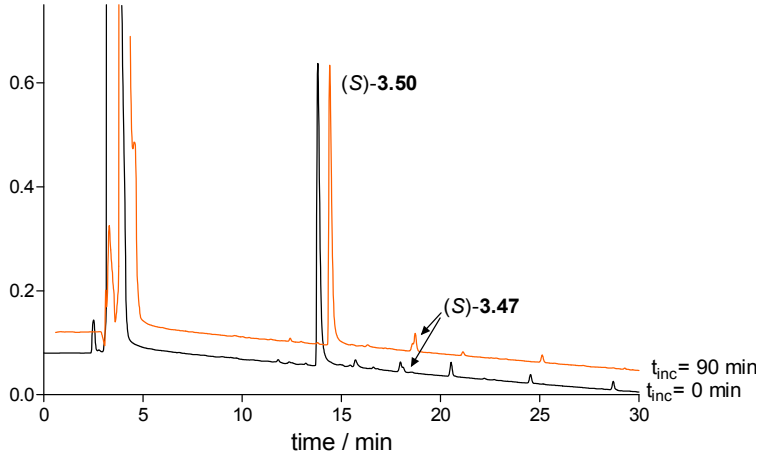

Figure 8.5. HPLC analysis of the Y_2R antagonist (*S*)-**3.50** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

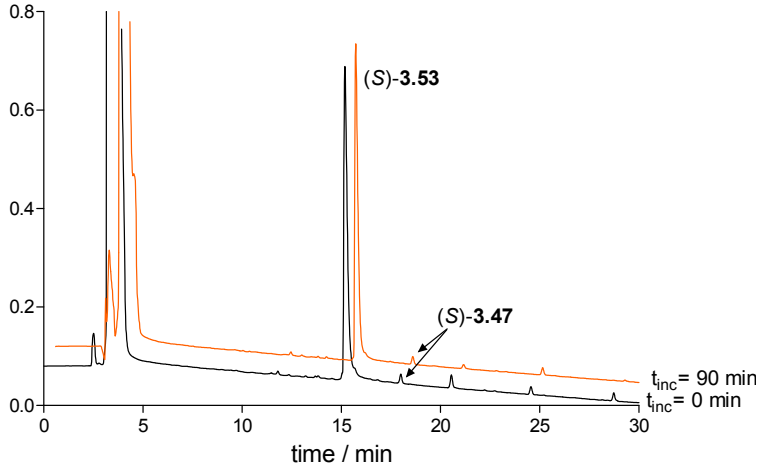

Figure 8.6. HPLC analysis of the Y_2R antagonist (*S*)-**3.53** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

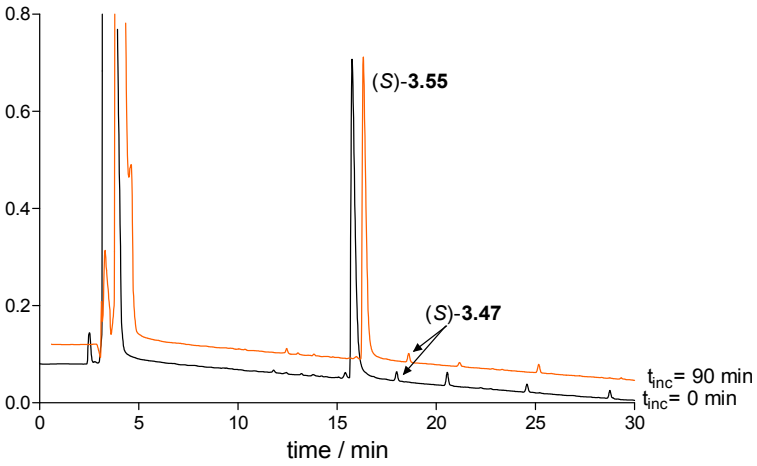

Figure 8.7. HPLC analysis of the Y_2R antagonist (*S*)-**3.55** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

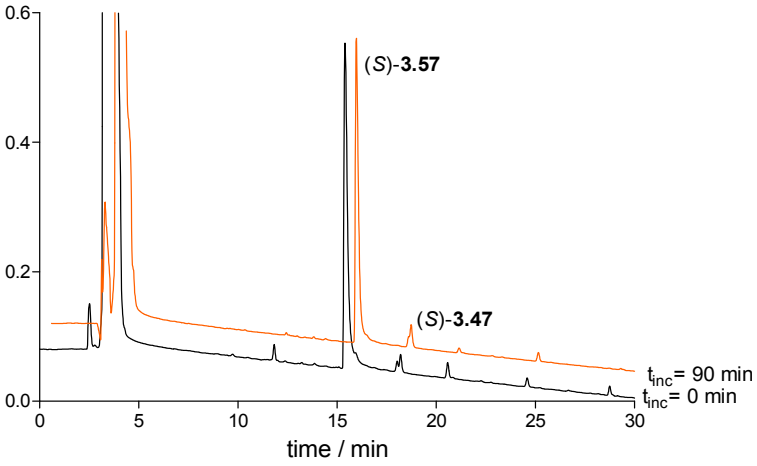

Figure 8.8. HPLC analysis of the Y_2R antagonist (*S*)-**3.57** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

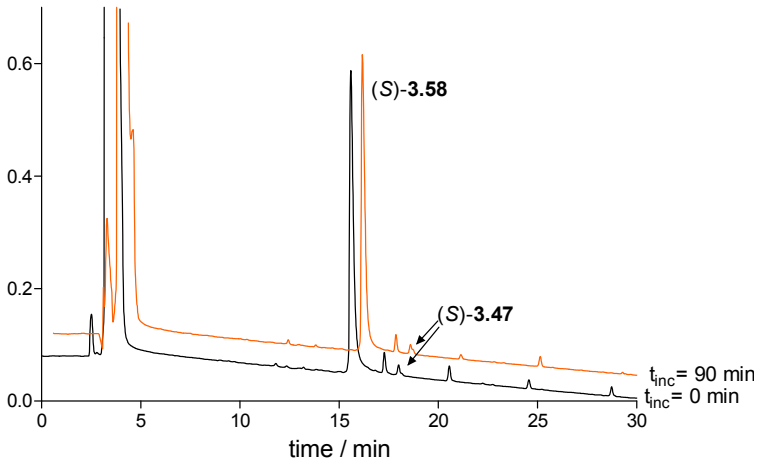

Figure 8.9. HPLC analysis of the Y_2R antagonist (*S*)-**3.58** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

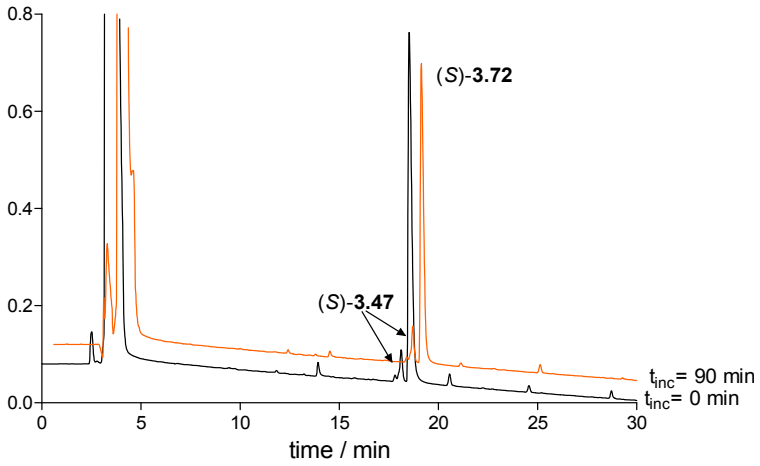

Figure 8.10. HPLC analysis of the Y_2R antagonist (*S*)-**3.72** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

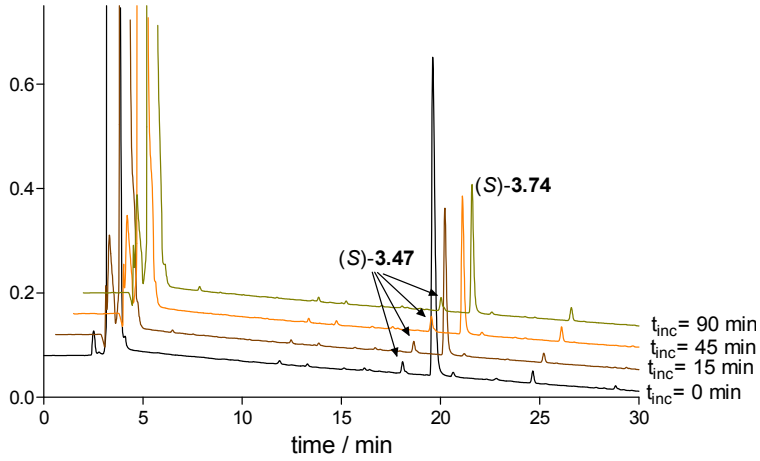

Figure 8.11. HPLC analysis of the Y_2R antagonist (*S*)-**3.74** in buffer. BIIE 0246 ((*S*)-**3.47**) is formed by decomposition of the ligand. Incubation: 90 min in buffer, pH 7.4, 20 °C. Conditions: eluent: mixtures of acetonitrile (A) and 0.05 % aq. TFA (B), gradient: 0 to 30 min: A/B 20/80 to 95/5.

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Regensburg, im September 2011

(Nikola Pluym)