Bioorganic & Medicinal Chemistry 18 (2010) 6292–6304

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

N^G-Acyl-argininamides as NPY Y_1 receptor antagonists: Influence of structurally diverse acyl substituents on stability and affinity

Stefan Weiss [a], Max Keller [b], Günther Bernhardt [b], Armin Buschauer [b], Burkhard König [a,*]

[a] Institut für Organische Chemie, Universität Regensburg, D-93040 Regensburg, Germany
[b] Institut für Pharmazie, Universität Regensburg, D-93040 Regensburg, Germany

ARTICLE INFO

Article history:
Received 16 February 2010
Revised 3 July 2010
Accepted 10 July 2010
Available online 16 July 2010

Keywords:
Acyl guanidines
NPY
BIBP 3226
GPCR

ABSTRACT

N^G-Acylated argininamides, covering a broad range of lipophilicity (calculated log *D* values: −1.8–12.5), were synthesized and investigated for NPY Y_1 receptor (Y_1R) antagonism, Y_1R affinity and stability in buffer (N^G-deacylation, yielding BIBP 3226). Broad structural variation of substituents was tolerated. The K_i (binding) and K_b values (Y_1R antagonism) varied from low nM to one-digit μM. Most of the compounds proved to be sufficiently stable at pH 7.4 over 90 min to determine reliable pharmacological data in vitro. Exceptionally high instability was detected when a succinyl moiety was attached to the guanidine, probably, due to an intramolecular cleavage mechanism.

1. Introduction

G Protein-coupled receptors (GPCRs) represent a major class of biological targets in drug discovery. Focusing on neuropeptide Y (NPY) and histamine receptors as models of aminergic and peptidergic GPCRs, respectively, we are particularly interested in bioisosteric approaches to develop special receptor subtype-selective tools, for example, bivalent ligands, prodrugs, radiolabeled and fluorescent compounds.[1–6] Strongly basic groups such as guanidines (including Arg residues) are essential structural features of numerous GPCR ligands, but are unfavourable with respect to bioavailability and brain penetration. This is also true for many high affinity NPY and histamine receptor agonists and antagonists.[7,8] Therefore, special effort was put into the search for bioisosteric replacements of strongly basic functional groups. Recently, we reported on the exploitation of guanidine–acylguanidine bioisosterism with respect to histamine H_2, H_3 and H_4 receptor ligands[5,9–12] and arginine-type NPY Y_1 receptor antagonists.[1,3,4,6,7,13,14]

NPY is a highly conserved peptide which plays an important role as a neurotransmitter in the central and peripheral nervous system.[15] In humans, four receptor subtypes, referred to as NPY Y_1, Y_2, Y_4 and Y_5 receptors, mediate the biological effects of NPY. For instance, in the periphery NPY Y_1 receptor (Y_1R) stimulation causes an increase in blood pressure. In the central nervous system (CNS) Y_1R activation elicits anxiolytic and sedative effects and is involved in the stimulation of food intake.

Arginine derivatives such as the Y_1R antagonist BIBP 3226[16] (**1a**, Fig. 1) have been proven as valuable pharmacological tools regardless of properties being far from drug-like. Interestingly, the reduction of the basicity of the Arg-derived Y_1R antagonist **1a** by introducing electron-withdrawing N^G-substituents such as acyl groups turned out to be a promising general route to guanidine derivatives with increased potency, to fluorescent ligands and radioactive tracers.[3–7,14] Even bulky fluorophores attached to the guanidine via spacers of different size were tolerated; a moderate decrease in affinity did not compromise suitability of the compounds as pharmacological tools. However, depending on the chemical nature of the linker cleavage of the acylguanidine group may occur as demonstrated for a model compound.[17]

In continuation of our studies on the structure–activity relationships of argininamide-type Y_1R antagonists we synthesized a series of N^G-acylated analogues of **1a** in order to explore the impact of structurally diverse substituents on stability and pharmacological activity in vitro (Y_1R binding and antagonism). As the small library of Y_1R antagonists was synthesized to cover a wide range of distribution coefficients (log *D*) the BIBP 3226 skeleton was substituted with hydrophobic alkyl chains or sugars and amines to alter the polarity of the parent compound (Fig. 1).

2. Results and discussion

2.1. Synthesis of the N^G-acylated argininamides

The N^G-acylated argininamides were efficiently prepared according to the general synthetic route shown in Scheme 1.

* Corresponding author. Tel.: +49 941 943 4576; fax: +49 941 943 1717.
E-mail address: Burkhard.koenig@chemie.uni-regensburg.de (B. König).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.07.028

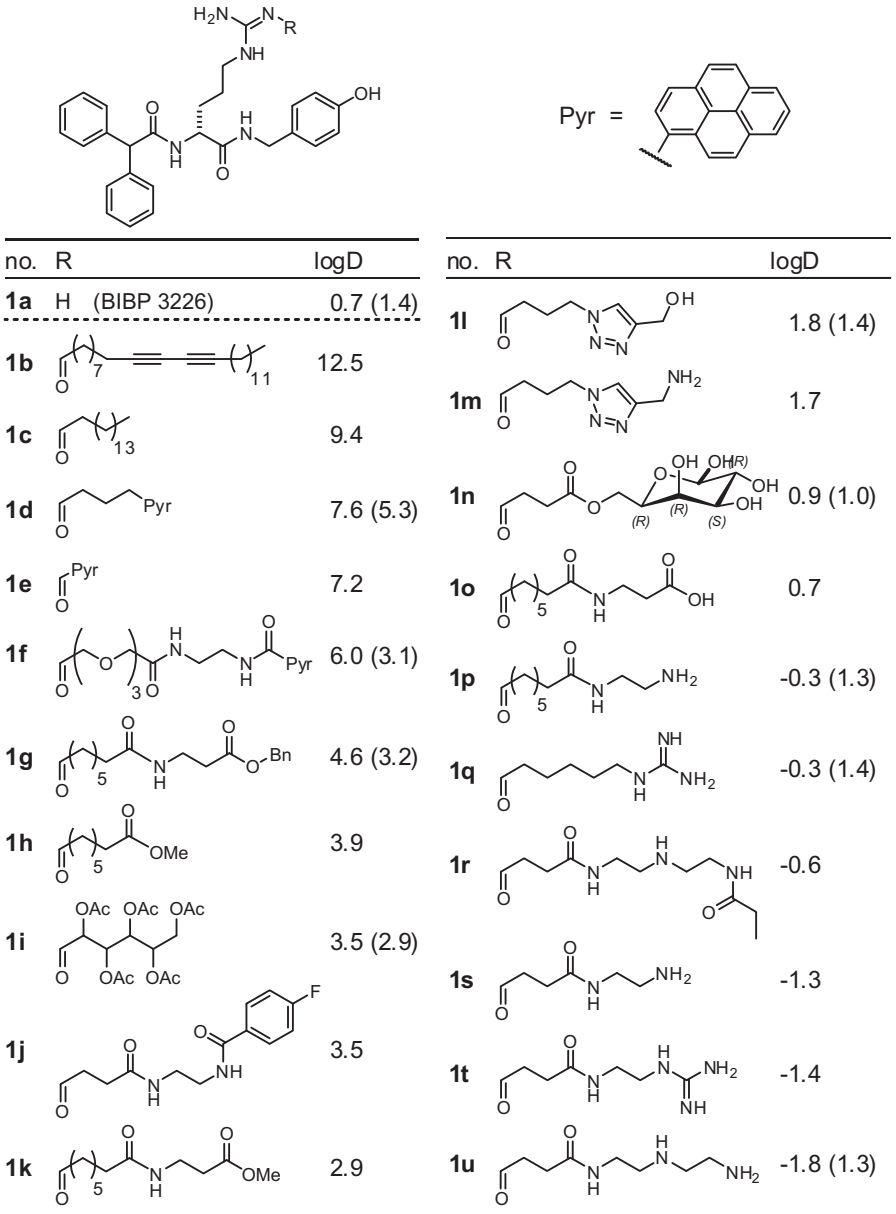
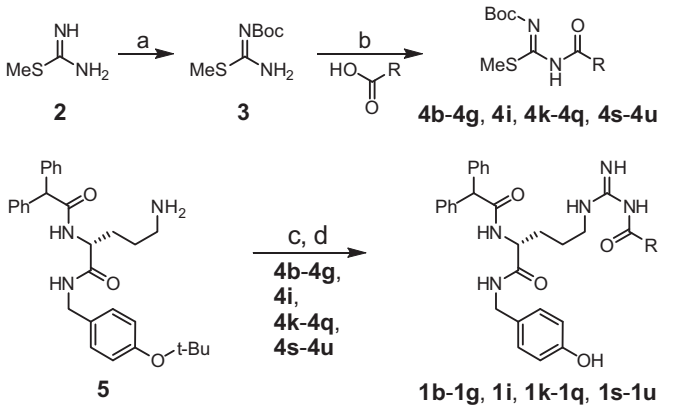


Figure 1. Structures of N^G-acylated derivatives of the Y_1R antagonist BIBP 3226 (**1a**) ranked according to calculated log *D* values (ACD-labs software version 12, pH 7.4); experimental log *D* values from HPLC measurements[18] in parentheses.

N-Boc-*S*-methylisothiourea (**3**) was acylated with the respective carboxylic acids yielding the isothiourea derived guanidinylation reagents **4b–4g**, **4i**, **4k–4q**, **4s–4u**. Guanidinylation of D-ornithinamide **5** and subsequent removal of protecting groups gave the envisaged N^G-acylated argininamides. Amine **5** is available from D-ornithine in six steps in 32% overall yield.[4]

Synthetic strategies for the preparation of carboxylic acids **7**, **8**, **11**, **14**, **17**, **20**, **22**, **27**, **28**, **30** and **36** are depicted in Schemes 2–4. Acids **7** and **8**, containing a triazole entity, were prepared through a Cu(I)-catalysed 'click-reaction' between 4-azidobutanoic acid and ^{t}Bu-protected propargyl alcohol or Boc-protected propargyl amine (Scheme 2). Carboxylic acid **14** was prepared via guanidinylation of 6-aminohexanoic acid methyl ester with *N*,*N*′-diBoc-*S*-methylisothiourea followed by ester hydrolysis (Scheme 2). For the synthesis of succinic acid derivative **17** ethylenediamine was onefold guanidinylated with *N*,*N*′-diBoc-*S*-methylisothiourea yielding amine **16**, which was treated with succinic anhydride (Scheme 2). Acid precursors **20** and **22** were obtained through the treatment of amines **19** and **21** with succinic anhydride (Scheme 2). The suberic acid derivatives **27** and **28** were prepared through amidation of octandioic acid mono-benzyl ester with amines **24** and **21**, respectively, followed by benzyl ester cleavage (Scheme 3). Octandioic acid derivative **30** was obtained via amidation of non-protected suberic acid with 3-aminopropanoic acid benzyl ester (Scheme 3). The synthesis of carboxylic acid **36** started from pyrene-1-carboxylic acid (**31**), which was amidated with mono-Boc-protected ethylenediamine (**21**) in moderate yield (Scheme 4). The Boc group was removed with hydrochloric acid yielding amine **33**, which was coupled with 3,6,9-trioxaundecandioic acid monobenzyl ester (**34**) to give compound **35**. Hydrogenolysis of **35** resulted in acid **36** (Scheme 4).

D-Gluconic acid derivative **1i** was synthesized from penta-acetylated D-gluconic acid. Unfortunately, deprotection of the hydroxyl groups failed. The base-labile acylguanidine moiety prevents basic selective cleavage of the acetyl protecting groups. Enzymatic deprotection using a lipase preparation (Novozym 435) was also



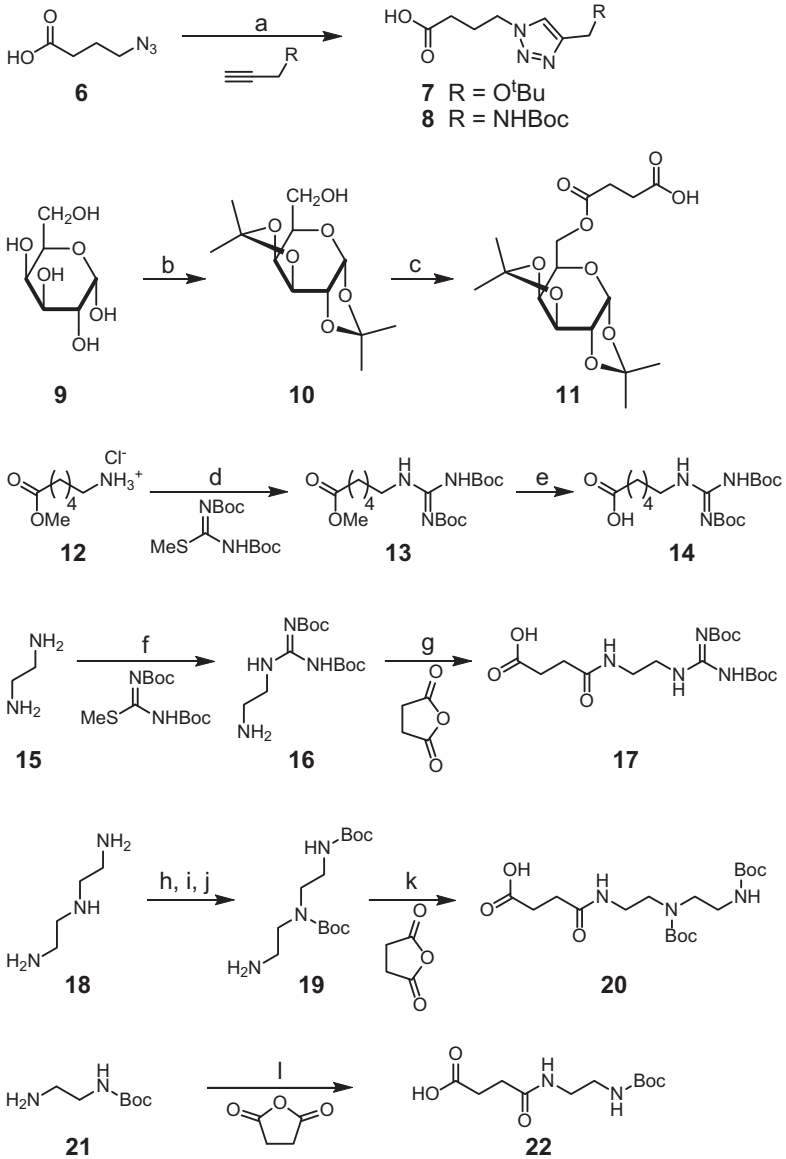
Scheme 1. General route for the synthesis of N^G-acylated BIBP 3226 derivatives **1b**–**1g**, **1i**, **1k**–**1q** and **1s**–**1u**. Reagents and conditions: (a) Boc_2O, NaOH, ^{t}BuOH, 90%; (b) DIPEA, EDC, HOBT, DCM, (**1s**: TBTU, DIPEA, DMF), 31–95%; (c) $HgCl_2$, NEt_3, DMF, 39–78%; (d) TFA/DCM 1:1, quantitative yield.

unsuccessful. To circumvent such problems, sugar derivative **1n**, containing a galactose entity, was prepared. The synthesis of the respective carboxylic acid **11** is shown in Scheme 2. Two acetal protecting groups were introduced in the first step and the remaining primary hydroxyl function was esterified with succinic anhydride. The acetal groups are compatible with the general synthetic strategy outlined in Scheme 1 and can be easily removed in the final deprotection step with TFA/DCM.

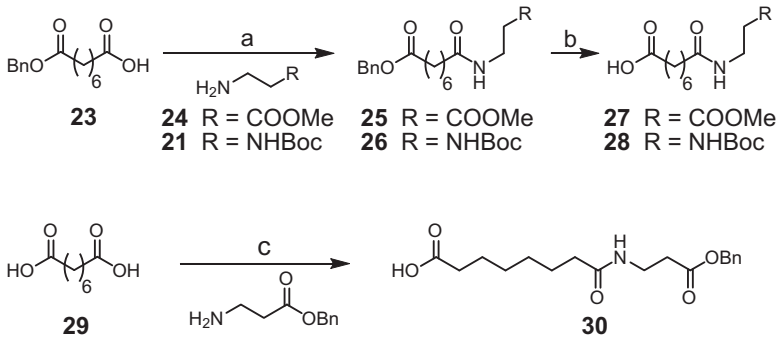
BIBP 3226 derivatives **1j** and **1r** were prepared through 4-fluorobenzoylation and propionylation of amines **1s** and **1u**, respectively (Scheme 5).

2.2. Stability and Y_1R antagonistic activity

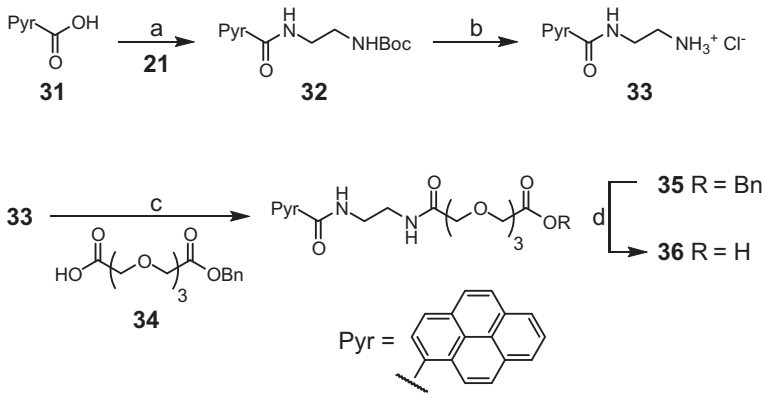
As the acylguanidine moiety of N^G-acylated argininamide-type Y_1R antagonists may be considered as the most probable cleavage site the stability of the presented BIBP 3226 (**1a**) derivatives was investigated with respect to the formation of **1a** at physiological pH of 7.4. A release of **1a** under the conditions of the pharmacolog-



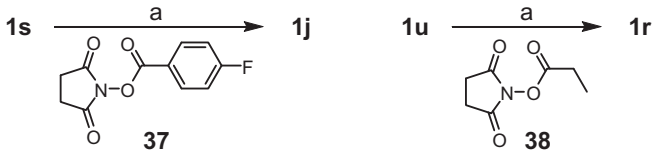
Scheme 2. Synthesis of carboxylic acids **7**, **8**, **11**, **14**, **17**, **20** and **22**. Reagents and conditions: (a) $CuSO_4$ (5 mol %), Na-ascorbate (10 mol %), MeOH, H_2O, 67–86%; (b) acetone, iodine, 71%; (c) succinic anhydride, DMAP, NEt_3, 73%; (d) $HgCl_2$, NEt_3, DMF, 84%; (e) THF, NaOH, 71%; (f) $CHCl_3$, 16 h, 97%; (g) THF, NEt_3, 49%; (h) CF_3COOEt, DCM; (i) Boc_2O, DCM; (j) K_2CO_3, MeOH, H_2O, 47% overall; (k) succinic anhydride, THF, NEt_3, 77%; (l) DCM, 99%.



Scheme 3. Synthesis of suberic acid derivatives **27**, **28** and **30**. Reagents and conditions: (a) DCM, EDC, HOBt, DIPEA, 62%; (b) Pd/C, H_2, 90–94%; (c) DCM, EDC, HOBt, DIPEA, 44%.



Scheme 4. Synthesis of the pyrene-1-carboxamide derivative **36**. Reagents and conditions: (a) DCM, EDC, HOBt, DIPEA, 52%; (b) MeOH, HCl, 83%; (c) DMF, EDC, HOBt, DIPEA, 59%; (d) Pd/C, MeOH, quantitative.



Scheme 5. Synthesis of the N^G-acylated BIBP 3226 derivatives **1j** and **1r**. Reagents and conditions: (a) NEt_3, DMF, 65% (**1j**), 21% (**1r**).

ical assays has to be taken into account as **1a** is a highly potent Y_1R antagonist (K_i = 1.3 nM) and could pretend a higher potency of the investigated compounds. Therefore the stability of the N^G-acylated argininamides was investigated at pH 7.4 on the time scale of the assays. Formation of **1a** was not observed for the strongly hydrophobic compounds **1c**, **1d** and **1e**, which are devoid of hetero atoms in their N^G-acyl substituents. The hydrophobic ligands **1f** and **1g**, both containing an amide group in the acyl substituent, showed a very minor decomposition over 90 min (<0.5%). Instability was more pronounced (decomposition up to 2.6% over 90 min) for the more polar compounds (**1h**, **1i**, **1k**–**1q**) bearing amide, ester, triazole, sugar, amine, carboxylic or guanidine functions in the acyl residue.

Exceptionally high instabilities were found for argininamides with succinyl attached to the guanidine (**1j**, **1r**–**1u**). Compounds **1r**–**1u** quantitatively decomposed to **1a** over 90 min (Table 1). This process was exemplarily explored by RP-HPLC–MS using the hydrophobic compound **1j**, which allows the detection of the acyl substituent upon cleavage. As becomes obvious from Figure 2 compound **1j** is cleaved via an intramolecular attack of the succinic amide nitrogen at the carbonyl group attached to the guanidine resulting in the *N*-alkyl succinimide derivative **39**. The lability of the acylguanidines bearing nucleophiles in position 5 or 6 of the acyl side chain is in accordance with the recently reported reactivity of 5-aminopentanoyl substituted guanidine.[17]

All compounds proved to be stable at acidic pH (0.1 % aqueous trifluoroacetic acid, pH 2–3). Under these conditions the acylguanidine group is almost quantitatively protonated due to pK_a values in the range of 7–8. Obviously, this changes the susceptibility against hydrolysis and prevents intramolecular attacks of nucleophiles at the carbonyl group, respectively.

Except for the highly unstable compounds **1j** and **1r**–**1u** the N^G-acylated argininamides were characterised in terms of Y_1R antagonism (K_b values) and Y_1R affinity (K_i values) using a Fura-2 assay on human erythroleukemia (HEL) cells[19] and a radioligand binding assay on SK-N-MC human neuroblastoma cells,[4] respectively. K_b and K_i values are summarised in Table 1. Except for **1h** (K_i = 0.9 nM) all N^G-acylated argininamides showed a reduced Y_1R affinity compared to the parent compound **1a** (K_i = 1.3 nM, Table 1). The decrease in affinity was most pronounced (up to three orders of magnitude) for the most hydrophobic compounds (**1b**–**1e**, Table 1), which have limited solubility and might interact with the cell membrane.

Direct attachment of pyrene-1-carboxylic acid to the guanidine-*N* (compound **1e**) resulted in complete loss of Y_1R affinity. By contrast, high Y_1R affinity can be retained, if the pyrene moiety is attached to the guanidine group through a linker (compounds **1d** and **1f**, Fig. 1, Table 1).

The Y_1R affinity of the polar sugar and guanidine substituted compounds **1n** and **1q** is about 35 times lower compared to the affinity of **1a**. The argininamide **1a** may be considered a mimic of the C-terminal dipeptide in NPY (...-Arg^{35}-Tyr^{36}-NH_2). It may be

Table 1
log *D* values, stability, Y_1R antagonistic activity (K_b) and Y_1R binding affinity (K_i) of BIBP 3226 derivatives **1b**–**1u**

No.	log *D*[a]	% Decomposition (pH 7.4, 20 °C) after 20/90 min	Y_1R Antagonism K_b[b] [nM]	Y_1R Affinity K_i[c] [nM]
1a[d]	0.7 (1.4)	—	1.5 ± 0.2	1.3 ± 0.2
1b	12.5	n.d.	140 ± 10	1500 ± 120
1c	9.4	0/0	170 ± 8	460 ± 19
1d	7.6 (5.3)	0/0	n.d.[e]	270 ± 70
1e	7.2	0/0	n.d.[e]	Inactive
1f	6.0 (3.1)	0/<0.5	n.d.[e]	59 ± 16
1g	4.6 (3.2)	0/<0.5	1.10 ± 0.04	40 ± 9
1h	3.9	0/0.8	0.06 ± 0.01[f]	0.9 ± 0.1[f]
1i	3.5 (2.9)	0.9/2.6	83 ± 8	110 ± 38
1j	3.5	30/69	n.d.[g]	n.d.[g]
1k	2.9	0/0.7	170 ± 18	260 ± 82
1l	1.8 (1.4)	0.9/2.1	0.40 ± 0.03	3.0 ± 0.5
1m	1.7	0.6/1.7	14 ± 4	6.7 ± 0.1
1n	0.9 (1.0)	<0.5/1.3	510 ± 190	41 ± 5
1o	0.7	<0.5/1.4	450 ± 52	73 ± 11
1p	−0.3 (1.3)	<0.5/1.4	230 ± 64	130 ± 10
1q	−0.3 (1.4)	<0.5/1.6	18 ± 8	51 ± 18
1r	−0.6	64/100	n.d.[g]	n.d.[g]
1s	−1.3	51/100	n.d.[g]	n.d.[g]
1t	−1.4	61/100	n.d.[g]	n.d.[g]
1u	−1.8 (1.3)	40/100	n.d.[g]	n.d.[g]

[a] Calculated with ACD-labs software version 12, pH 7.4; in brackets: experimental log *D* determined with HPLC measurements.
[b] K_b values for inhibition of NPY (10 nM) induced calcium mobilisation in HEL cells (Fura-2 assay); all mean values ± SEM from two or three (**1k**, **1n**, **1o**, **1p**, **1u**) independent experiments.
[c] K_i values determined from the displacement of 1.5 nM [^{3}H]-UR-MK114[4] on SK-N-MC cells; all mean values ± SEM from two or three (**1c**, **1f**, **1g**, **1k**, **1l**, **1p**) independent experiments.
[d] BIBP 3226.
[e] Due to their fluorescent properties pyrene ligands are not compatible with the Fura-2 assay.
[f] Keller et al.[1]
[g] Not determined due to the high instability.

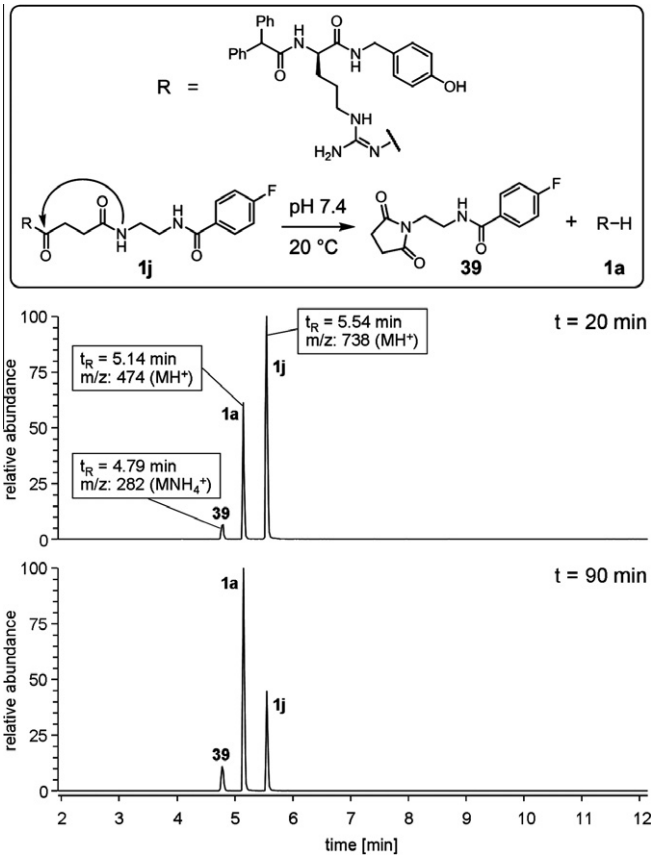


Figure 2. Exploration of the decomposition mechanism of BIBP 3226 derivative **1j** incubated in an aqueous buffer (pH 7.4) at 20 °C. LC–MS analysis was performed after an incubation period of 20 and 90 min. Compound **1j** is decomposed to BIBP 3226 (**1a**) and the succinimide derivative **39** through an intramolecular attack of the succinic amide nitrogen at the guanidine linked carbonyl group. Chromatograms were acquired by single ion monitoring analysis.

speculated that the introduction of a second guanidine moiety (as provided by compound **1q**) could enhance binding affinity through mimicking the second arginine residue (Arg^{33}).[20] However, the K_i value of compound **1q** was only 51 nM—perhaps due to an inappropriate distance between the two guanidine groups.

The hydroxy substituted Y_1R antagonist **1l** (K_i = 3.0 nM), proved to be as potent as **1a**. It is conceivable that the hydroxy group mimics Thr^{32} or Tyr^{27} of the natural ligand NPY. Both amino acids are very important for the binding of NPY to the Y_1R, as identified by alanine scan.[21] The amine analogue of **1l**, compound **1m**, binds with similar affinity to the Y_1R (K_i = 6.7 nM).

As partial decomposition of compounds **1i**, **1k** and **1o**–**1q** occurs (up to 2.6% over 90 min), the determined K_i values of these five compounds have to be considered with reservation, as the affinity of the cleavage product **1a** (K_i = 1.3 nM) and the assay periods (from preparation of solutions to read out: 60–90 min) have to be taken into account.

With respect to the prediction of the affinity of new compounds, three issues have to be taken into account: substitution with bulky groups directly at the BIBP 3226 backbone results in a complete loss of affinity that can be compensated with a spacer of adequate length. Derivatives with a small aromatic or heteroaromatic substituent (**1g**, **1l**, **1m**) show slightly higher binding affinity compared to the more flexible derivatives (**1g** vs **1k**, **1o**, **1p**; **1m** vs **1q**). Relatively small hydrophobic substituents, as in **1h** (K_i = 0.9 nM) and the radioligand [^{3}H]-UR-MK114 (K_i = 1.3 nM),[4] result in similar and even higher affinity compared to BIBP 3226. Interactions of the acyl substituent with the Y_1R are difficult to predict, because these residues are presumably oriented toward flexible extracellular loop regions[7] and, depending on chemical nature and size of the acyl residue, an interaction with membrane lipids cannot be ruled out.

3. Conclusion

The stability of the N^G-acylated argininamides strongly depends on the nature of the acyl residue. It becomes obvious from the high instability of the succinyl derivatives (**1j**, **1r**–**1u**) that cleavage is favoured when the residues harbour nucleophilic functional groups capable of an intramolecular attack on the acyl carbonyl. This is also conceivable for argininamides decomposing to a minor extent and bearing N^G-acyl residues containing amide, ester, triazole, sugar, amine, carboxylic or guanidine functions (**1h**, **1i**, **1k**–**1q**). This has to be taken into account in pharmacological investigations, in particular, when cleavage products are highly bioactive as in the case of **1a** (K_i = 1.3 nM). However, despite these limitations broad structural variation of N^G-acyl substituents was tolerated (affinities in the nM range). Most of the investigated compounds proved to be sufficiently stable at pH 7.4 to determine reliable in vitro pharmacological data. In conclusion, the N^G-acylation of the stongly basic guanidine group in argininamides is a successful bioisosteric approach to more drug-like properties provided that the outlined stability considerations are taken care of. This is not restricted to the NPY field, as guanidine groups are crucial structural features of many different biologically active compounds including GPCR ligands.

4. Experimental

4.1. General experimental conditions

Unless otherwise noted, solvents (analytical grade) were purchased from commercial suppliers and used without further purification. Ethyl acetate (EA), petrol ether (PE, 60–70 °C), methanol and dichloromethane were obtained in technical grade and distiled before application. Acetonitrile (MeCN) for HPLC was obtained from Merck (Darmstadt, Germany). Pentacosa-10,12-diynoic acid (Sigma–Aldrich Chemie GmbH, Munich, Germany), 4-(pyren-1-yl)butanoic acid (Sigma–Aldrich Chemie GmbH, Munich, Germany), hexadecanoic acid (Riedel-de Haen, Seelze, Germany), suberic acid (Fluka, Sigma–Aldrich Chemie GmbH, Munich, Germany) and D-(+)-galactose (Merck, Darmstadt, Germany) were purchased. Preparative HPLC was performed with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps and a K-2001 detector. A Nucleodur 100-5 C18 (250 × 21 mm, 5 μm; Macherey-Nagel, Germany) and a Eurospher-100 C18 (250 × 32 mm, 5 μm; Knauer, Germany) served as RP-columns at flow rates of 20 and 38 mL/min, respectively. Mixtures of MeCN and diluted aqueous TFA (0.1 %) were used as mobile phase. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker Avance 300 spectrometer or at 600 MHz on a Bruker Avance III 600 with cryogenic probehead (Bruker, Karlsruhe, Germany). ^{13}C NMR spectra were recorded at 75 MHz on a Bruker Avance 300 spectrometer. All chemical shifts values are reported in ppm. UV/Vis spectra were recorded with a Varian Cary BIO 50 UV/Vis/NIR spectrophotometer (Varian Inc., CA, USA). Mass spectra: Finnigan SSQ 710A (EI), Finnigan MAT 95 (CI), Finnigan MAT TSQ 7000 (Thermo FINNIGAN, USA) (ES/LC–MS). LC-system for LC–MS: Agilent 1100 (Palo Alto, USA). LC–MS method I (LC–MS-I): column: Phenomenex Luna C18, 3.0 μm, 100 × 2 mm HST (Phenomenex, Aschaffenburg, Germany); flow: 0.30 mL/min; solvent A (water + 0.1% TFA), solvent B (MeCN); gradient: 0 min [A/B 95:5], 1 min [A/B 95:5], 11 min [A/B 2:98], 18 min [A/B 2:98], 19 min [A/B 95:5], 24 min [A/B 95:5]. LC–MS method II: column: Phenomenex Luna C18, 2.5 μm, 50 × 2 mm HST (Phenomenex, Aschaffenburg, Germany); flow: 0.40 mL/min; solvent A (water + 0.1% TFA), solvent B (MeCN); gradient: 0 min [A/B 95:5], 8 min [A/B 2:98], 11 min [A/B 2:98], 12 min [A/B 95:5], 15 min [A/B 95:5]. Analytical HPLC (HPLC): Compounds **1c**–**1i**, **1k**–**1q**, **1t**, **1u**: Phenomenex Luna C18, 3.0 μm, 150 × 2 mm (Phenomenex, Aschaffenburg, Germany); flow: 0.30 mL/min; solvent A (H_2O + 0.0059%TFA), solvent B (MeCN); gradient: 0 min [A/B 95:5], 30 min [A/B 2:98]; **1j**, **1r**, **1s**: Eurospher-100 C18, 5 μm, 250 × 4.0 mm (Knauer, Berlin, Germany); flow: 0.80 mL/min; solvent A (water + 0.05% TFA), solvent B (MeCN); gradient: 0 min [A/B 85:15], 28 min [A/B 45:55], 33 min [A/B 5:95], 40 min [A/B 5:95]. Melting points were determined with a Lambda Photometrics Optimelt MPA100 apparatus (Lambda photometrics, Harpenden, UK), they are not corrected. Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck Silica Gel 60 F_{245}, thickness 0.2 mm). Column chromatography (CC) was performed with Merck Geduran SI 60 Silica Gel as the stationary phase.

The synthesis of **38**, **1a** and **1h** was described elsewhere.[1,4] Compounds **3**,[2] **5**,[4] **6**,[22] penta-acetyl gluconic acid,[23] ^{t}Bu-protected propargyl alcohol,[24] ^{t}Bu-protected propargyl amine,[25] **10**,[26] **11**,[27] **14**,[28] **16**,[29] **19**,[30] **21**,[31] **23**,[32] **31**,[33] **37**[4] were prepared according to literature procedures.

4.2. Synthetic protocols and analytical data of 7, 8, 17, 20, 22, 25–28, 30 and 32–36

4.2.1. 4-[4-(*tert*-Butoxymethyl)-1*H*-1,2,3-triazol-1-yl]butanoic acid (7)

4-Azidobutanoic acid (419 mg, 3.25 mmol) was mixed with Boc-protected propargyl alcohol (364 mg, 3.25 mmol) in 5 mL MeOH. Then ascorbic acid (65 mg, 0.33 mmol) dissolved in 1 mL H_2O and copper sulfate pentahydrate (8 mg, 0.03 mmol) dissolved in 1 mL H_2O were added and the reaction mixture was heated to reflux overnight. Next day MeOH was evaporated completely and dichloromethane (30 mL) and satd aqueous $NaHSO_4$ solution (20 mL) were added. The organic layer was collected and then diluted NaOH (40 mL, 1 mol/L) was added. The aqueous layer was collected and acidified with $NaHSO_4$ solution until pH <2. Dichloromethane (40 mL) was added and the acid was extracted. The organic phase was dried over $MgSO_4$, the solvent was evaporated and a white solid was obtained (674 mg, 86%), mp 81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.26 (s, 9H), 2.15–2.25 (m, 2H), 2.39 (t, J = 6.88, 2H), 4.42 (t, J = 6.94, 2H), 4.58 (s, 2H), 7.55 (s, 1H), 10.31 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 25.3, 27.5, 30.5, 49.3, 56.2, 74.1, 122.5, 146.8, 176.4. $C_{11}H_{19}N_3O_3$: MS (CI, NH_3): *m/z* (%) 242 (100, MH+).

4.2.2. 4-{4-[(*tert*-Butoxycarbonylamino)methyl]-1*H*-1,2,3-triazol-1-yl}butanoic acid (8)

4-Azidobutanoic acid (0.89 g, 6.90 mmol) was mixed with Boc-protected propargylamine (1.07 g, 6.90 mmol) in MeOH (10 mL). NaOH (1 mol/L) was added until the pH value was between 6 and 8. Then ascorbic acid (65 mg, 0.33 mmol) dissolved in 1 mL H_2O and copper sulfate pentahydrate (8 mg, 0.03 mmol) dissolved in 1 mL H_2O were added and the reaction mixture was heated to reflux overnight. Next day MeOH was evaporated under reduced pressure and the residue was diluted with 80 mL EA and 80 mL of aqueous $NaHSO_4$ solution (5%w). The organic layer was separated, dried over $MgSO_4$ and the solvent was evaporated. The crude material was recrystallised from EA yielding a white crystalline solid (1.31 g, 67%), mp 117 °C. ^{1}H NMR (300 MHz, CD_3OD): 1.43 (s, 9H), 2.10–2.20 (m, 2H), 2.25–2.35 (m, 2H), 4.29 (s, 2H), 4.44 (t, J = 6.89, 2H), 7.82 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): 26.7, 29.0, 31.5, 36.9, 50.6, 80.5, 124.2, 147.3, 158.3, 176.1. $C_{12}H_{20}N_4O_4$: MS (LC–MS-II): *m/z* (%) [t_R = 5.1 min]: 285 (35, MH+), 569 (100).

4.2.3. 6-(*tert*-Butoxycarbonylamino)-2,2-dimethyl-4,11-dioxo-3-oxa-5,7,10-triaza-tetradec-5-en-14-oic acid (17)

Compound **16** (300 mg, 0.99 mmol) and succinic anhydride (119 mg, 1.19 mmol) were dissolved in a mixture of THF (10 mL) and NEt_3 (150 mg, 1.49 mmol) and stirred overnight. Next day water was added (10 mL) and the mixture was stirred for 1 h. THF was evaporated completely and the residue was diluted with water (20 mL). Aqueous $NaHSO_4$ solution (5%w, 10 mL) was added and the mixture was extracted with dichloromethane (2 × 50 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated yielding a white solid (197 mg, 49%), mp 77–80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (s, 9H), 1.49 (s, 9H), 2.50–2.60 (m, 2H), 2.60–2.70 (m, 2H), 3.35–3.45 (m, 2H), 3.45–3.60 (m, 2H), 7.00–8.40 (br s, 1H), 8.50 (br s, 1H), 8.67 (br s, 1H), 11.38 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 28.0, 28.2, 30.3, 30.5, 40.3, 41.9, 79.9, 83.9, 153.0, 157.6, 162.5, 172.9, 175.1. $C_{17}H_{30}N_4O_7$: MS (LC–MS-I): *m/z* (%) [t_R = 10.3 min]: 403 (100, MH+), 805 (10).

4.2.4. 4-{2-[(2-*tert*-Butoxycarbonylaminoethyl)(*tert*-butoxycarbonyl)amino]ethylamino}-4-oxobutanoic acid (20)

Compound **19** (1.00 g, 3.30 mmol) was dissolved in 5 mL THF and a solution of succinic anhydride (0.33 g, 3.30 mmol) in 5 mL THF was added. Then NEt_3 (0.50 g, 4.95 mmol) was added to the mixture. The mixture was stirred overnight at ambient temperature. Next day THF was removed completely and the crude product was dissolved in 20 mL dichloromethane. It was washed with aqueous $NaHSO_4$ soln (5%w, 30 mL), dried over $MgSO_4$ and the solvent was evaporated giving a white solid (1.03 g, 77%), mp 90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.40 (s, 9H), 1.43 (s, 9H), 2.48 (br s, 2H), 2.64 (br s, 2H), 3.10–3.40 (m, 8H), 4.95–5.25 (m, 1H),

7.00–7.30 (m, 1H), 9.31 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 20.4, 29.7, 30.6, 39.3, 39.6, 47.0, 47.8, 79.6, 80.7, 156.6, 156.7, 172.8, 175.7. $C_{18}H_{33}N_3O_7$: MS (ES): *m/z* (%) 204 (5), 245 (45), 304 (50), 348 (25), 404 (100, MH+), 426 (5), 808 (7), 825 (15).

4.2.5. 3-(2-*tert*-Butoxycarbonylaminoethyl)aminocarbonyl-propanoic acid (22)

Succinic anhydride (0.41 g, 4.06 mmol) and NEt_3 (56 μL, 0.41 mmol) were added to a solution of amine **21** (0.65 g, 4.06 mmol) in dichloromethane (3 mL). The mixture was heated to 60 °C for 25 min (microwave synthesizer (Biotage Initiator 8)). After removal of the solvent under reduced pressure the product (insoluble in dichloromethane) was afforded as a white solid (1.05 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6): 1.37 (s, 9H), 2.25–2.32 (m, 2H), 2.37–2.44 (m, 2H), 2.91–2.99 (m, 2H), 3.00–3.08 (m, 2H), 6.78 (t, J = 5.45, 1H), 7.85 (t, J = 5.29, 1H), 12.08 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 28.1, 29.0, 29.9, 38.6, 39.5, 77.5, 155.5, 171.0, 173.8. $C_{11}H_{20}N_2O_5$: (LC–MS-II): *m/z* (%) [t_R = 4.63 min]: 261 (40, MH+), 278 (6, MNH_4+), 521 (35, 2MH+), 538 (100, $2MNH_4$+).

4.2.6. Benzyl 8-(3-methoxy-3-oxopropylamino)-8-oxooctanoate (25)

Acid **23** (250 mg, 0.95 mmol), DIPEA (368 mg, 2.85 mmol), and HOBT·H_2O (142 mg, 1.05 mmol) were dissolved in ice-cold dichloromethane (20 mL) and EDC (147 mg, 0.95 mmol) was added under nitrogen atmosphere. After 15 min amine **24** (98 mg, 0.95 mmol) was added. The reaction mixture was stirred overnight. Next day the reaction mixture was washed with aqueous $NaHSO_4$ soln (5%w, 20 mL) and satd aqueous $NaHCO_3$ solution (20 mL). The solvent was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (PE/EA 3:1→EA R_f = 0.4 [EA]) yielding the product as a white solid (253 mg, 76%), mp 48 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.31 (m, 4H), 1.60 (m, 4H), 2.10 (m, 2H), 2.30 (t, J = 7.48, 2H), 2.50 (m, 2H), 3.50 (dd, J_1 = 6.07, J_2 = 11.99, 2H), 3.68 (s, 3H), 5.10 (s, 2H), 6.00–6.10 (br s, 1H), 7.30–7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): 24.0, 25.4, 28.75, 28.80, 33.9, 34.2, 34.7, 36.6, 51.8, 66.1, 128.2, 128.6, 136.1, 173.0, 173.2, 173.6. $C_{19}H_{27}NO_5$: MS (CI, NH_3): *m/z* (%) 350 (100, MH+).

4.2.7. Benzyl 8-[2-(*tert*-butoxycarbonylamino)ethylamino]-8-oxooctanoate (26)

Compound **23** (264 mg, 1.00 mmol) was dissolved in 10 mL of dichloromethane. HOBT·H_2O (149 mg, 1.10 mmol) and DIPEA (387 mg, 3.00 mmol) were added under nitrogen atmosphere. The mixture was stirred and cooled in an ice bath. EDC (171 mg, 1.10 mmol) was added and after 15 min amine **21** (160 mg, 1.00 mmol) was added. The mixture was stirred overnight. Next day DCM was added (10 mL), the organic phase was washed once with aqueous $NaHSO_4$ (5%w, 20 mL) and satd aqueous $NaHCO_3$ soln (20 mL), was dried over $MgSO_4$ and the organic layer was evaporated. The crude product was purified by column chromatography (PE/EA 1:1→EA R_f = 0.3 [EA]). A white, wax-like solid (251 mg, 62%), mp 60 °C was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): 1.25–1.25 (m, 4H), 1.43 (s, 9H), 1.55–1.70 (m, 4H), 2.10–2.17 (m, 2H), 2.30–2.37 (m, 2H), 3.20–3.30 (m, 2H), 3.30–3.40 (m, 2H), 4.97 (br s, 1H), 5.10 (s, 2H), 6.20 (br s, 1H), 7.30–7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): 24.7, 25.4, 28.4, 28.7, 28.8, 34.1, 36.5, 40.3, 40.7, 66.1, 79.6, 128.2, 128.6, 136.1, 157.0, 173.6, 173.8. $C_{22}H_{34}N_2O_5$: MS (ES): *m/z* (%) 407 (100, MH+).

4.2.8. 8-(3-Methoxy-3-oxopropylamino)-8-oxooctanoic acid (27)

Compound **25** (210 mg, 0.60 mmol) was dissolved in MeOH, placed into an autoclave and Pd/C (25 mg) was added after flushing the autoclave with N_2. The reaction mixture was stirred overnight at room temperature and 12 bar hydrogen pressure. Pd/C was removed by filtration over Celite, MeOH was evaporated and a white solid was obtained (146 mg, 94%), mp 73 °C. ^{1}H NMR (300 MHz, CD_3OD): 1.34 (m, 4H), 1.59 (m, 4H), 2.16 (t, J = 7.45, 2H,), 2.27 (t, J = 7.39, 2H), 2.52 (t, J = 6.64, 2H), 3.41 (t, J = 6.64, 2H), 3.67 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD): 26.0, 26.9, 29.90, 29.92, 34.8, 34.9, 36.4, 36.9, 52.2, 173.9, 176.4, 177.7. $C_{12}H_{21}NO_5$: MS (CI, NH_3): *m/z* (%) 260 (100, MH+), 277 (41).

4.2.9. 8-[2-(*tert*-Butoxycarbonylamino)ethylamino]-8-oxooctanoic acid (28)

Compound **26** (172 mg, 0.42 mmol) was dissolved in 5 mL MeOH. Pd/C (17 mg) was added and the mixture was stirred at 15 bar hydrogen pressure in an autoclave overnight. Pd/C was filtered off using Celite and MeOH was evaporated. The product was obtained as a white solid (120 mg, 90%), mp 95 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.15–1.25 (m, 4H), 1.31 (s, 9H), 1.40–1.55 (m, 4H), 2.00–2.10 (m, 2H), 2.10–2.20 (m, 2H), 3.00–3.10 (m, 2H), 3.10–3.17 (m, 2H), 4.05 (br s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 24.5, 25.3, 28.1, 28.5, 28.6, 33.9, 36.1, 39.6, 39.7, 79.6, 157.1, 174.9, 176.5. $C_{15}H_{28}N_2O_5$: MS (ES): *m/z* (%) 217 (100), 261 (45), 317 (85, MH+), 339 (15), 633 (10), 650 (25).

4.2.10. 8-[3-(Benzyloxy)-3-oxopropylamino]-8-oxooctanoic acid (30)

Octanedioic acid (500 mg, 2.87 mmol), DIPEA (1.11 g, 8.61 mmol) and HOBT·H_2O (427 mg, 3.16 mmol) were dissolved in ice-cold dichloromethane (20 mL) and EDC (490 mg, 3.16 mmol) was added under nitrogen atmosphere. After 15 min 3-aminopropanoic acid benzyl ester (617 mg, 2.87 mmol) was added and the ice bath was removed. The mixture was stirred overnight. Next day an aqueous $NaHSO_4$ solution (5%w, 20 mL) was added and the crude product was extracted with dichloromethane (2 × 20 mL), the organic phase was dried over $MgSO_4$, filtered and evaporated. The crude material was purified by column chromatography, (EA→EA/MeOH 9:1). The obtained material is a 2:1 mixture of the desired product (420 mg, 44%) and the appropriate octanedioic acid diamide. The mixture was used in the next synthesis step without further purification.

4.2.11. *tert*-Butyl 2-(pyrene-1-carbonylamino)ethylcarbamate (32)

Pyrene-1-carboxylic acid (246 mg, 1.00 mmol), DIPEA (387 mg, 3.00 mmol) and HOBT·H_2O (149 mg, 1.10 mmol) were dissolved in ice-cold dichloromethane (20 mL). EDC (171 mg, 1.10 mmol) was added under nitrogen atmosphere. After 15 min Boc-protected ethylenediamine (160 mg, 1.0 mmol) was added and the ice bath was removed. The mixture was stirred overnight. Next day the reaction mixture was diluted with dichloromethane (40 mL), washed with aqueous $NaHSO_4$ solution (5%w, 40 mL) and satd aquoeus $NaHCO_3$ solution (1 × 40 mL). The organic layer was dried over $MgSO_4$ and evaporated. The crude compound was recrystallised from EA yielding the product as a yellow solid (213 mg, 55%), mp >190 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.41 (s, 9H), 3.20–3.30 (m, 2H), 3.40–3.50 (m, 2H), 7.00 (t, J = 5.57, 1H), 8.05–8.30 (m, 5H), 8.30–8.40 (m, 3H), 8.50–8.54 (m, 1H), 8.71 (t, J = 5.37, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 28.2, 39.58, 39.61, 77.6, 123.5, 123.7, 124.2, 124.7, 125.3, 125.5, 125.7, 126.5, 127.1, 127.9, 128.2, 130.1, 130.6, 131.5, 131.8, 155.7, 168.9. $C_{24}H_{24}N_2O_3$: MS (EI): *m/z* (%) 201 (58), 229 (100), 245 (57), 258 (15), 389 (22, MH+).

4.2.12. 2-(Pyren-1-carbonylamino)ethanaminium chloride (33)

Compound **32** (549 mg, 1.41 mmol) was suspended in a mixture of 20 mL MeOH and 5 mL HCl (37%). It was stirred 30 min at 70 °C. The solvent was evaporated completely and a yellow solid was obtained (445 mg, 97%), mp >190 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 3.05–3.20 (m, 2H), 3.65–3.75 (m, 2H), 8.05–8.15 (m, 1H), 8.15–

8.30 (m, 3H), 8.30–8.35 (m, 4H), 8.35–8.50 (br s, 3H), 8.62 (m, 1H), 9.02 (t, J = 5.33, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 37.3, 38.4, 123.5, 123.7, 124.2, 124.7, 125.6, 125.7, 125.8, 126.5, 127.1, 128.0, 128.3, 130.0, 130.6, 130.8, 131.7, 169.2. $C_{19}H_{16}N_2O$: MS (CI, NH_3): m/z (%) 289 (100, MH+).

4.2.13. 3-Oxo-1-phenyl-2,5,8,11-tetraoxatridecan-13-oic acid (34)

3,6,9-Trioxaundecanedioic acid (5.55 g, 25 mmol), benzyl alcohol (2.70 mg, 25 mmol) and toluene sulfonic acid (43 mg, 0.25 mmol) were combined in a 250 mL flask and toluene (70 mL) was added. The flask was connected to a Dean–Stark apparatus. The mixture was heated to reflux and the reaction was completed after 1 h. satd aqueous $NaHCO_3$ (50 mL) solution was added and the phases were separated. The aqueous layer was collected and extracted with EA (3 × 100 mL). The organic layer was dried over $MgSO_4$ and evaporated. The product is a viscous oil (1.87 g, 24%). ^{1}H NMR (300 MHz, CD_3OD): 3.60–3.75 (m, 8H), 4.10 (s, 2H), 4.20 (s, 2H), 5.17 (s, 2H), 7.30–7.40 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD): 66.7, 68.5, 68.6, 70.3, 70.5, 70.7, 70.9, 128.44, 128.47, 128.52, 128.63, 128.65, 135.3, 170.5, 173.2. $C_{15}H_{20}O_7$: MS (CI, NH_3): m/z (%) 242 (6), 286 (28), 313 (2, MH+), 330 (100).

4.2.14. Benzyl 1,6-dioxo-1-(pyren-1-yl)-8,11,14-trioxa-2,5-diazahexadecan-16-oate (35)

Compound **34** (462 mg, 1.48 mmol), DIPEA (476 mg, 3.69 mmol) and HOBT·H_2O (200 mg, 1.48 mmol) were dissolved in DMF (10 mL) and EDC (229 mg, 1.48 mmol) was added (ice bath). After 15 min compound **33** (400 mg, 1.23 mmol) was added and the mixture was stirred overnight. Next day DMF was removed completely, the residue was dissolved in dichloromethane (20 mL) and washed with aqueous $NaHSO_4$ solution (5%w, 20 mL), satd aqueous $NaHCO_3$ solution (1 × 20 mL) and brine (1 × 20 mL). The crude product was purified with column chromatography (PE/EA 1:1→EA/MeOH 4:1 R_f = 0.3 [EA/MeOH 9:1]). The product is a yellow solid (420 mg, 59%), mp >190 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.45–3.80 (m, 12H), 3.97 (s, 2H), 4.02 (s, 2H), 5.07 (s, 2H), 7.20–7.40 (m, 6H), 7.64 (t, J = 5.57, 1H), 7.95–8.10 (m, 6H), 8.14–8.19 (m, 2H), 8.55–8.60 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 38.8, 40.9, 66.6, 68.4, 70.1, 70.27, 70.32, 70.7, 70.9, 124.3, 124.4, 124.6, 124.69, 124.7, 125.65, 125.72, 126.3, 127.1, 128.39, 128.47, 128.51, 128.55, 128.62, 130.7, 130.95, 131.12, 132.4, 135.2, 170.1, 170.4, 171.4. $C_{34}H_{34}N_2O_7$: MS (ES): m/z (%) 583 (100, MH+), 605 (60), 621 (10). UV (MeOH): λ (ε) 233 (65 × 10^3), 242 (89 × 10^3), 265 (34 × 10^3), 275 (51 × 10^3), 326 (31 × 10^3), 340 (41 × 10^3). Fluorescence (MeOH): λ (type) 383 (monomer), 401 (monomer)

4.2.15. 1,6-Dioxo-1-(pyren-1-yl)-8,11,14-trioxa-2,5-diazahexadecan-16-oic acid (36)

Compound **35** (203 mg, 0.35 mmol) was dissolved in 5 mL of MeOH. Pd/C (10 mg) was added and the mixture was stirred in an autoclave at 1 bar hydrogen pressure for 4 h. After that time Pd/C was filtered off with Celite and the solvent was removed. The product is a yellow solid (172 mg, 100%), mp >190 °C. ^{1}H NMR (300 MHz, CD_3OD): 3.50–3.58 (m, 4H), 3.58–3.65 (m, 4H), 3.65–3.75 (m, 4H), 4.01 (s, 2H), 4.03 (s, 2H), 8.00–8.30 (m, 8H), 8.45–8.55 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 38.1, 39.2, 67.5, 69.4, 69.6, 69.7, 69.9, 70.2, 123.5, 123.7, 124.3, 124.7, 125.2, 125.5, 125.7, 126.5, 127.1, 127.7, 128.0, 128.2, 130.1, 130.6, 131.5, 131.8, 169.0, 169.6, 171.6. $C_{27}H_{28}N_2O_7$: MS (ES): m/z (%) 493 (100, MH+), 510 (10), 515 (50).

4.3. General procedure for the preparation of compounds 4b–4g, 4i, 4k–4q, 4t, 4u

The appropriate carboxylic acid (1 mmol), HOBT·H_2O (1.2 mmol), EDC (1.2 mmol) and DIPEA (2 mmol) were combined in a flask under nitrogen atmosphere with 10 mL of cold DMF. After 15 min compound **3** (1 mmol) was added and the mixture was stirred overnight at room temperature. Next day the solvent was removed completely and the crude material was purified with column chromatography.

4.3.1. *tert*-Butyl (pentacosa-10,12-diynamido)methylthiomethylenecarbamate (4b)

Column chromatography (PE/EA 9:1, R_f = 0.3). The product is a white solid (489 mg, 89%), mp 37 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.75–0.85 (t, J = 7.24, 3H), 1.10–1.70 (m, 41H), 2.17 (t, J = 6.92, 4H), 2.30–2.45 (m, 5H), 12.00–12.45 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.13, 14.45, 19.18, 19.20, 22.69, 24.50, 27.99, 28.29, 28.36, 28.74, 28.86, 28.91, 29.06, 29.11, 29.35, 29.49, 29.62, 29.64, 29.65, 31.92, 37.38, 65.25, 65.34, 77.35, 77.54, 81.17, 160.97, 171.32, 171.49. $C_{32}H_{54}N_2O_3S$: MS (LC–MS-I): m/z (%) [t_R = 15.3 min]: 547 (100, MH+).

4.3.2. *tert*-Butyl (hexadecanamido)methylthiomethylenecarbamate (4c)

Column chromatography (PE/EA 9:1 R_f = 0.5). The product is a white solid (342 mg, 80%), mp 62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.82 (t, J = 6.68, 3H), 1.18–1.28 (m, 24H), 1.46 (s, 9H), 1.60 (m, 2H), 2.33 (s, 3H), 2.37 (m, 2H), 12.40 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.1, 14.5, 28.0, 22.7, 29.1–29.7, 31.9, 80.2. $C_{23}H_{44}N_2O_3S$: MS (CI, NH_3): m/z (%) 329 (15), 429 (100, MH+).

4.3.3. *tert*-Butyl-[4-(pyren-1-yl)butanamido]methylthiomethylenecarbamate (4d)

Column chromatography (PE/EA 9:1→PE/EA 3:1, R_f = 0.4 [PE/EA 3:1]). The product was obtained as a white solid (299 mg, 65%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$): 1.53 (s, 9H), 2.18–2.32 (m, 2H), 2.32–2.43 (m, 3H), 2.45–2.70 (m, 2H), 3.41 (t, J = 7.46, 2H), 7.80–7.90 (m, 1H), 7.95–8.05 (m, 3H), 8.05–8.20 (m, 4H), 8.25–8.35 (m, 1H), 12.15–12.55 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 26.1, 28.1, 32.5, 36.7, 81.3, 123.3, 124.86, 124.97, 125.01, 125.10, 125.88, 126.76, 127.32, 127.43, 127.47, 127.53, 128.74, 130.02, 130.03, 131.43, 135.26, 150.98, 161.04, 171.24. $C_{27}H_{28}N_2O_3S$: MS (LC–MS-I): m/z (%) [t_R = 15.5 min]: 461 (100, MH+).

4.3.4. *tert*-Butyl-(pyren-1-carbonylamino)methylthiomethylenecarbamate (4e)

Column chromatography (PE/EA 9:1, R_f = 0.1). The product was obtained as a bright yellow solid (190 mg, 45%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$): 1.54–1.63 (m, 9H), 2.53–2.67 (m, 3H), 7.95–8.40 (m, 7H), 8.90–9.00 (m, 1H), 9.40–9.50 (m, 1H), 12.60–13.40 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 15.1, 28.1, 83.6, 124.03, 124.36, 124.99, 125.55, 126.07, 126.21, 127.27, 127.38, 129.09, 129.54, 129.67, 130.42, 130.47, 131.05, 131.25, 134.25, 151.25, 171.15, 178.73. $C_{24}H_{22}N_2O_3S$: MS (LC–MS-I): m/z (%) [t_R = 16.3 min]: 419 (100, MH+), 837 (10).

4.3.5. *tert*-Butyl 5,15,20-trioxo-20-(pyren-1-yl)-7,10,13-trioxa-2-thia-4,16,19-triazaicosan-3-ylidenecarbamate (4f)

Column chromatography (EA→EA/MeOH 9:1, R_f = 0.3 [EA/MeOH 9:1]). The product was obtained as a light yellow solid (485 mg, 73%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$): 1.49 (s, 9H), 2.20–2.40 (m, 3H), 3.50–3.75 (m, 10H), 3.75–3.85 (m, 2H), 3.90–4.05 (m, 4H), 7.10–7.90 (m, 2H), 7.95–8.15 (m, 6H), 8.15–8.25 (m, 2H), 8.55–8.65 (m, 1H), 11.80–12.90 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 28.0, 39.0, 40.9, 70.4, 70.5, 70.6, 70.8, 70.9, 71.5, 77.2, 124.4, 124.6, 124.7, 125.7, 125.8, 126.3, 127.1, 128.6, 128.7, 130.7, 131.2, 132.5, 165.9, 169.1, 170.4. $C_{34}H_{40}N_4O_8S$: MS (ES): m/z (%) 565 (50), 665 (100, MH+), 687 (15).

4.3.6. Benzyl 3-{8-[(Boc-amino)-methylthio-methyleneamino]-8-oxooctanamido}propanoate (4g = 4o)[†]

Column chromatography (PE/EA 1:1→PE/EA 3:7, R_f = 0.15[PE/EA 1:1]). The product is a viscous oil (364 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.25–1.40 (m, 4H), 1.51 (s, 9H), 1.55–1.70 (m, 4H), 2.05–2.15 (m, 2H), 2.35–2.50 (m, 2H), 2.38 (s, 3H), 2.55–2.60 (m, 2H), 3.45–3.55 (m, 2H), 5.13 (s, 2H), 6.02 (s, 1H), 7.30–7.40 (m, 5H), 12.45 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 25.4, 28.0, 28.7, 34.1, 34.8, 36.6, 66.6, 77.3, 128.3, 128.4, 128.7, 135.6, 172.6, 172.9. $C_{25}H_{37}N_3O_6S$: MS (ES): *m/z* (%) 408 (25), 508 (100, MH+).

4.3.7. (2*R*,3*R*,4*S*,5*R*)-6-[(Boc-amino)-methylthio-methyleneamino]-6-oxohexane-1,2,3,4,5-pentayl pentaacetate (4i)

Column chromatography (PE/EA 3:1, R_f = 0.1). Compound **4i** (252 mg, 44%) was obtained as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.48 (s, 9H), 2.00–2.10 (m, 12H), 2.23 (s, 3H), 2.42 (s, 3H), 4.19 (dd, J_1 = 5.84, J_2 = 12.33, 1H), 4.34 (dd, J_1 = 3.53, J_2 = 12.33, 1H), 5.05 (dt, J_1 = 3.53, J_2 = 5.84, 1H), 5.40 (d, J = 3.08, 1H), 5.52 (dd, J = 5.84, 1H), 5.90 (dd, J_1 = 3.08, J_2 = 5.84, 1H), 11.88 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.8, 20.5, 20.6, 20.7, 20.8, 21.1, 27.9, 61.5, 69.1, 69.3, 70.1, 74.5, 84.1, 150.5, 169.5, 169.7, 169.8, 170.2, 170.5, 174.5, 176.8. $C_{23}H_{34}N_2O_{13}S$: MS (ES): *m/z* (%) 579 (100, MH+).

4.3.8. Methyl 3-{8-[boc-amino(methylthio)methyleneamino]-8-oxooctanamido}propanoate (4k)

Column chromatography (PE/EA 1:1→EA, R_f = 0.4 [EA]). The product is a colourless oil (337 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.33 (m, 4H), 1.51 (s, 9H), 1.63 (m, 4H), 2.14 (m, 2H), 2.35–2.50 (m, 5H), 2.53 (m, 2H), 3.47–3.54 (dd, J_1 = 6.07, J_2 = 11.94, 2H), 3.70 (s, 3H), 6.04 (br s, 1H), 12.00–12.70 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 24.3, 25.4, 28.0, 28.7, 28.8, 33.9, 34.7, 36.6, 37.3, 51.8, 77.2, 81.3, 171.3, 172.9, 173.3. $C_{19}H_{33}N_3O_6S$: MS (CI, NH_3): *m/z* (%) 332 (14), 432 (100, MH+).

4.3.9. *tert*-Butyl-{4-[4-(*tert*-butoxymethyl)-1*H*-1,2,3-triazol-1-yl]butanamido}methylthio-methylenecarbamate (4l)

Column chromatography (PE/EA 3:1→PE/EA 4:6, R_f = 0.4[PE/EA 4:6]). Compound **4l** (254 mg, 62%) was obtained as a white solid, mp 93 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.26 (s, 9H), 1.49 (s, 9H), 2.15–2.30 (m, 2H), 2.37 (s, 3H), 2.40–2.60 (m, 2H), 4.40 (t, J = 6.33, 2H), 4.57 (s, 2H), 7.52 (s, 1H), 12.10–12.50 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 25.2, 27.6, 28.0, 35.3, 49.1, 56.5, 73.8, 81.5, 83.6, 122.1, 147.0, 171.0, 171.4. $C_{18}H_{31}N_5O_4S$: MS (CI): *m/z* (%) 314 (18), 414 (100, MH+).

4.3.10. *tert*-Butyl {4-[4-(Boc-aminomethyl)-1*H*-1,2,3-triazol-1-yl]butanamido}methylthio-methylenecarbamate (4m)

Column chromatography (PE/EA 3:1→PE/EA 4:6, R_f = 0.3[PE/EA 4:6]). Compound **4m** (341 mg, 75%) was obtained as a colourless, viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.34 (s, 9H), 1.41 (s, 9H), 2.10–2.25 (m, 2H), 2.29 (s, 3H), 2.30–2.50 (m, 2H), 4.25–4.40 (m, 4H), 5.20–5.40 (br s, 1H), 7.49 (s, 1H), 11.70–12.50 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 24.9, 27.9, 28.3, 33.9, 37.6, 49.4, 79.5, 122.0, 145.6, 155.9, 160.7, 169.9, 184.5. $C_{19}H_{32}N_6O_5S$: MS (LC–MS-II): *m/z* (%) [t_R = 7.3 min]: 457 (100, MH+), 913 (40).

4.3.11. ((3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-2,2,7,7-Tetramethyltetrahydro-3a*H*-bis[1,3]dioxolo[4,5-b:4′,5′-d]pyran-5-yl)methyl 4-((boc-amino)-methylthio-methyleneamino)-4-oxobutanoate (4n)

Column chromatography (PE/EA 9:1→PE/EA 3:1, R_f = 0.2 [PE/EA 9:1]). Compound **4n** (396 mg, 74%) was obtained as a colourless, viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.29 (d, J = 2.47, 6H), 1.40 (s, 3H), 1.42–1.50 (m, 12H), 2.34 (s, 3H), 2.60–2.85 (m, 4H), 3.90–4.00 (m, 1H), 4.19 (dd, J_1 = 1.55, J_2 = 7.85, 2H), 4.24 (d, J = 4.96, 1H), 4.28 (dd, J_1 = 2.50, J_2 = 4.99, 1H), 4.57 (dd, J_1 = 2.46, J_2 = 7.88, 1H), 5.48 (d, J = 4.98, 1H), 12.05–12.49 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 24.5, 24.9, 25.9, 26.0, 27.9, 28.3, 29.3, 31.8, 35.8, 63.5, 63.8, 65.9, 70.4, 70.7, 71.0, 81.3, 83.4, 96.3, 108.7, 109.6, 150.9, 169.8, 170.9, 171.8, 172.8, 184.3. $C_{23}H_{36}N_2O_{10}S$: MS (ES): *m/z* (%) 533 (100, MH+).

4.3.12. *tert*-Butyl [17,17-dimethyl-13-(methylthio)-4,11,15-trioxo-16-oxa-3,12,14-triazaoctadec-13-en-1-yl]carbamate (4p)

Column chromatography (PE/EA 1:1→PE/EA 3:7, R_f = 0.1 [PE/EA 1:1]). The product was obtained as a white solid (368 mg, 77%), mp 95 °C ^{1}H NMR (300 MHz, $CDCl_3$): 1.25–1.35 (m, 4H), 1.37 (s, 9H), 1.40–1.50 (m, 9H), 1.50–1.65 (m, 4H), 2.05–2.15 (m, 2H), 2.32 (s, 3H), 2.32–2.50 (m, 2H), 3.15–3.35 (m, 4H), 4.80–4.95 (br s, 1H), 6.00–6.20 (br s, 1H), 12.00–12.50 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 24.3, 25.4, 28.0, 28.4, 28.6, 28.8, 36.5, 37.2, 40.3, 40.8, 79.7, 81.3, 157.0, 161.0, 171.3, 171.5, 173.7. $C_{22}H_{40}N_4O_6S$: MS (ES): *m/z* (%) 389 (18), 489 (100, MH+).

4.3.13. *tert*-Butyl-16,16-dimethyl-5,14-dioxo-15-oxa-2-thia-4,11,13-triazaheptadecane-3,12-diylidenedicarbamate (4q)

Column chromatography (PE/EA 3:1, R_f = 0.35) Compound **4q** (413 mg, 76%) was obtained as a white solid, mp 116–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.30–1.45 (m, 2H), 1.48 (s, 9H), 1.49 (s, 9H), 1.52 (s, 9H), 1.55–1.75 (m, 4H), 2.30–2.55 (m, 5H), 3.35–3.45 (m, 2H), 8.30 (br s, 1H), 11.49 (br s, 1H), 12.16–12.48 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 24.2, 26.3, 28.0, 28.1, 28.3, 28.8, 37.1, 40.6, 79.2, 81.3, 83.1, 153.3, 156.1, 161.0, 163.6, 171.2, 171.3. $C_{24}H_{43}N_5O_7S$: MS (LC–MS-I): *m/z* (%) [t_R = 14.2 min]: 546 (100, MH+), 1092 (10).

4.3.14. Di-*tert*-butyl-17,17-dimethyl-5,8,15-trioxo-16-oxa-2-thia-4,9,12,14-tetraaza-octadecane-3,13-diylidenedicarbamate (4t)

Column chromatography (PE/EA 4:6, R_f = 0.2). The product was obtained as a colourless, viscous oil (180 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.43–1.52 (m, 27H), 2.34 (s, 3H), 2.50–2.90 (m, 4H), 3.35–3.75 (m, 4H), 7.60–7.90 (m, 1H), 8.40–8.70 (m, 1H), 11.37 (br s, 1H), 11.75–12.50 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 27.99, 28.04, 28.25, 38.6, 38.8, 40.4, 41.6, 79.6, 83.2, 83.6, 153.0, 153.1, 156.9, 157.4, 162.8, 177.5. $C_{24}H_{42}N_6O_8S$: MS (LC–MS-I): *m/z* (%) [t_R = 12.9 min]: 575 (100, MH+), 1149 (15).

4.3.15. *tert*-Butyl 14-boc-amino-12-boc-5,8-dioxo-2-thia-4,9,12-triazatetradecan-3-ylidenecarbamate (4u)

Column chromatography (PE/EA 1:1→EA, R_f = 0.15 [PE/EA 1:1]). The product was obtained as a colourless, viscous oil (386 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.40 (s, 9H), 1.45 (s, 9H), 1.48 (s, 9H), 2.35 (s, 3H), 2.40–2.90 (m, 4H), 3.10–3.50 (m, 8H), 4.85–5.10 (m, 1H), 6.50–7.00 (m, 1H), 11.70–12.60 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 28.0, 28.2, 28.3, 28.4, 39.0, 39.6, 47.2, 47.9, 79.4, 80.5, 156.1, 156.7, 170.5, 171.2. $C_{25}H_{45}N_5O_8S$: MS (ES): *m/z* (%) 576 (100, MH+).

4.3.16. *tert*-Butyl 11-boc-amino-5,8-dioxo-2-thia-4,9-diazaundecan-3-ylidenecarbamate (4s)

DIPEA (0.37 g, 2.84 mmol) and TBTU (0.96 g, 3.0 mmol) were added to a solution of **22** (0.74 g, 2.84 mmol) in DMF (5 mL). The mixture was stirred under argon for 15 min prior to the addition of **3** (0.595 g, 3.13 mmol) and DIPEA (0.74 g, 2.84 mmol). After stirring over night glacial acetic acid (162 μL, 2.84 mmol) was added and the solvent was removed under reduced pressure (1 mbar) at a temperature of 45 °C. Purification with column chromatography (dichloromethane/EA 3:1→1:2, R_f = 0.45[dichloromethane/EA

[†] This compound was used for preparation of **1g** and **1o**.

3:1]) yielded the product as a white solid (1.17 g, 95%). ^{1}H NMR (300 MHz, DMSO-d_6): 1.37 (s, 9H), 1.42 (s, 9H), 2.27 (2.25) (s, 3H), 2.30–2.38 (m, 2H), 2.53–2.59 (m, 2H), 2.09–2.99 (m, 2H), 2.99–3.10 (m, 2H), 6.75–6.90 (m, 1H), 7.86–7.99 (m, 1H), 11.24 (10.98) (s, 1H). $C_{18}H_{32}N_4O_6S$: (LC–MS-II): *m/z* (%) [t_R = 6.87 min]: 433 (100, MH+).

4.4. General procedure for the preparation of compounds 1b–1g, 1i, 1k–1q and 1s–1u

The appropriate *S*-methylisothiourea derivative **4** (0.2 mmol), compound **5** (0.2 mmol) and $HgCl_2$ (0.2 mmol) were dissolved separately in small amounts of DMF (1–2 mL). The solutions of the compounds **4** and **5** were combined in a small flask under nitrogen atmosphere. NEt_3 (2 mmol) was added under stirring, then the $HgCl_2$ solution was added and the mixture was stirred overnight at room temperature. Next day DMF was removed completely and the residue was dissolved in DCM. The precipitate of mercury salts was filtered off and the organic phase was concentrated. The crude products were purified by column chromatography (PE/EA mixtures). The Boc- and ^{t}Bu-protected target molecules were deprotected to the corresponding target compounds **1b**–**1g**, **1i**, **1k**–**1q** and **1s**–**1u** without further characterisation: The corresponding protected compound was dissolved in a dichloromethane/TFA 1:1 mixture (5 mL) and stirred for 2 h. After that time the solvent was removed completely under reduced pressure and the oily residue was repeatedly dissolved in dichloromethane and the solvent evaporated to remove TFA. Except for **1b** and **1s** (**1b**: purified by column chromatography, **1s**: no purification after deprotection) the deprotected compounds were purified with preparative HPLC. The given yield corresponds to the coupling step in the first part of this procedure, the deprotection step was quantitative.

4.4.1. (*R*)-1-[4-(2,2-Diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]-2-pentacosa-10,12-diynoylguanidinium 2,2,2-trifluoroacetate (1b)

The product is a light yellow solid (170 mg, 72%), mp >190 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6): 0.85 (t, *J* = 6.51, 3H), 1.00–1.80 (m, 36H), 2.26 (t, *J* = 6.67, 4H), 2.41 (t, *J* = 7.19, 2H), 3.15–3.30 (m, 2H), 4.00–4.25 (m, 2H), 4.30–4.40 (m, 1H), 5.12 (s, 1H), 6.67 (d, *J* = 8.43, 2H), 7.00 (d, *J* = 8.41, 2H), 7.15–7.35 (m, 10H), 8.00–9.70 (m, 6H), 11.50–11.80 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 13.8, 18.2, 22.0, 23.73, 27.57, 27.59, 28.05, 28.06, 28.15, 28.27, 28.45, 28.60, 28.75, 28.83, 28.90, 31.19, 36.02, 52.15, 55.80, 65.23, 77.84, 77.90, 114.87, 126.47, 128.04, 128.07, 128.29, 128.38, 128.40, 129.00, 140.15, 140.34, 152.71, 156.15, 170.86, 170.91, 175.17. $C_{52}H_{71}N_5O_4$: MS (LC–MS-I): *m/z* (%) [t_R = 12.3 min]: 830 (100, MH+).

4.4.2. (*R*)-1-[4-(2,2-Diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]-2-hexadecanoylguanidinium 2,2,2-trifluoracetate (1c)

The product is a white solid (48 mg, 53%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$): 0.88 (t, 3H), 1.20–1.35 (m, 26H), 1.40–1.70 (m, 4H), 2.42 (t, 2H), 2.70–3.10 (m, 2H), 3.90–4.10 (m, 1H), 4.15–4.35 (m, 2H), 4.99 (s, 1H), 5.80–6.50 (br s, 1H), 6.60 (d, 2H), 6.90 (d, 2H), 7.15–7.30 (m, 10H), 7.40–7.90 (m, 3H), 8.87 (br s, 1H), 9.45 (br s, 1H), 12.13 (br s, 1H), $C_{43}H_{61}N_5O_4$: MS (ES): *m/z* (%) 712 (100, MH+). HPLC: 100% (ELSD, t_R = 23.8 min).

4.4.3. (*R*)-1-[4-(2,2-Diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]-2-[4-(pyren-1-yl)butanoyl]guanidinium 2,2,2-trifluoroacetate (1d)

The product is a white solid (82 mg, 74%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.80 (m, 4H), 2.00–2.15 (m, 2H), 2.55–2.65 (t, *J* = 6.98, 2H), 3.15–3.25 (m, 2H), 3.35–3.45 (m, 2H), 4.05–4.20 (m, 2H), 4.25–4.40 (m, 1H), 5.12 (s, 1H), 6.67 (d, *J* = 8.48, 2H), 7.00 (d, *J* = 8.47, 2H), 7.10–7.30 (m, 10H), 7.90–8.00 (m, 1H), 8.00–8.10 (m, 1H), 8.15 (br s, 2H), 8.20–8.35 (m, 4H), 8.35–8.65 (m, 5H), 8.80–8.95 (m, 1H), 9.31 (br s, 1H), 11.32 (br s, 1H). $C_{47}H_{45}N_5O_4$: MS (LC–MS-I): *m/z* (%) [t_R = 10.2 min]: 744 (100, MH+). HPLC: 100% (ELSD, t_R = 19.1 min). UV (MeCN): λ (ε) 264 (20×10^3), 275 (31×10^3), 312 (10×10^3), 326 (17×10^3), 342 (23×10^3). Fluorescence ($\lambda_{ex.}$ = 340 nm, MeCN): 397 nm (monomer), 419 nm (monomer).

4.4.4. (*R*)-1-[4-(2,2-Diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]-2-(pyrene-1-carbonyl)guanidinium 2,2,2-trifluoroacetate (1e)

The product is a yellow solid (106 mg, 68%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, CD_3CN): 1.50–1.90 (m, 4H), 3.20–3.40 (m, 2H), 4.10–4.30 (m, 2H), 4.35–4.45 (m, 1H), 5.05 (s, 1H), 6.72 (d, *J* = 8.51, 2H), 7.04 (d, *J* = 8.47, 2H), 7.15–7.40 (m, 13H), 8.05–8.40 (m, 8H), 8.50–8.60 (m, 1H), 9.25–10.30 (m, 2H), 13.05 (s, 1H). $C_{44}H_{39}N_5O_4$: MS (LC–MS-I): *m/z* (%) [t_R = 9.7 min]: 702 (100, MH+). HPLC: 99% (ELSD, t_R = 17.8 min). UV (MeCN): λ (ε) 242 (45×10^3), 277 (33×10^3), 348 (28×10^3). Fluorescence ($\lambda_{ex.}$ = 340 nm, MeCN): 406 nm (very broad).

4.4.5. (*R*)-23-(4-Hydroxybenzylcarbamoyl)-1,6,16,25-tetraoxo-26,26-diphenyl-1-(pyren-1-yl)-8,11,14-trioxa-2,5,17,19,24-pentaazahexacosan-18-iminium 2,2,2-trifluoroacetate (1f)

The product is a white solid (52 mg, 46%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.80 (m, 4H), 3.10–3.80 (m, 14H), 3.94 (s, 2H), 4.05–4.20 (m, 2H), 4.19 (s, 2H), 4.28–4.38 (m, 1H), 5.12 (s, 1H), 6.67 (d, *J* = 8.45, 2H), 7.00 (d, *J* = 8.45, 2H), 7.15–7.30 (m, 10H), 7.98 (t, *J* = 5.65, 1H), 8.08–8.45 (m, 9H), 8.45–8.55 (m, 2H), 8.55–8.85 (m, 3H), 8.95–9.05 (m, 1H), 9.32 (br s, 1H), 10.61 (br s, 1H). $C_{54}H_{57}N_7O_9$: MS (ES): *m/z* (%) 474 (88), 948 (100, MH+). HPLC: 99% (ELSD, t_R = 16.3 min). UV (H_2O): λ (ε) 267 (26×10^3), 277 (35×10^3), 330 (24×10^3), 343 (30×10^3). Fluorescence ($\lambda_{ex.}$ = 340 nm, H_2O): λ (type) 401 (monomer).

4.4.6. (*R*)-2-{8-[3-(Benzyloxy)-3-oxopropylamino]-8-oxooctanoyl}-1-[4-(2,2-diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]guanidinium 2,2,2-trifluoroacetate (1g)

The product is a white solid (58 mg, 78%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.00–1.80 (m, 12H), 1.95–3.50 (m, 10H), 4.05–4.20 (m, 2H), 4.25–4.40 (m, 1H), 5.08 (s, 2H), 5.12 (s, 1H), 6.67 (d, *J* = 8.36, 2H), 7.00 (d, *J* = 8.40, 2H), 7.20–7.40 (m, 15H), 7.85–7.95 (m, 1H), 8.30–8.60 (m, 4H), 8.70–8.90 (br s, 1H), 9.31 (br s, 1H), 11.20 (br s, 1H). $C_{45}H_{54}N_6O_7$: MS (ES): *m/z* (%) 791 (100, MH+), 1582 (1). HPLC: 100% (ELSD, t_R = 16.2 min).

4.4.7. (4*R*,12*R*,13*S*,14*R*,15*R*)-12,13,14,15-Tetraacetoxy-4-(2,2-diphenylacetamido)-1-(4-hydroxyphenyl)-3,11,18-trioxo-17-oxa-2,8,-10-triazanonadecan-9-iminium 2,2,2-trifluoroacetate (1i)

The product is a white solid (197 mg, 66%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.70 (m, 4H), 1.90–2.20 (m, 15H), 3.20–3.30 (m, 2H), 4.00–4.40 (m, 5H), 5.00–5.10 (br s, 1H), 5.12 (s, 1H), 5.24 (br s, 1H), 5.35–5.45 (m, 1H), 5.45–5.55 (m, 1H), 6.67 (d, *J* = 8.45, 2H), 7.00 (d, *J* = 8.45, 2H), 7.15–7.35 (m, 10H), 8.36 (t, *J* = 5.58, 1H), 8.47 (d, *J* = 8.14, 1H), 8.50–8.90 (m, 2H), 9.30 (br s, 1H), 11.80 (br s, 1H). $C_{43}H_{51}N_5O_{14}$: MS (ES): *m/z* (%) 862 (100, MH+), 884 (60). HPLC: 100% (ELSD, t_R = 16.2 min).

4.4.8. (*R*)-21-(4-Hydroxybenzylcarbamoyl)-3,7,14,23-tetraoxo-24,24-diphenyl-2-oxa-6,15,17,22-tetraazatetracosan-16-iminium 2,2,2-trifluoroacetate (1k)

The product is a white solid (149 mg, 64%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$): 1.10–1.70 (m, 12H), 2.16 (t, *J* = 7.28, 2H), 2.35 (t, *J* = 6.51, 2H), 2.54 (t, *J* = 5.66, 2H), 2.95 (br

s, 1H), 3.15 (br s, 1H), 3.50 (m, 2H), 3.69 (s, 3H), 4.00–4.35 (m, 3H), 4.98 (s, 1H), 6.48 (t, J = 5.40, 1H), 6.70 (d, J = 7.86, 2H), 6.94 (d, J = 7.82, 2H), 7.15–7.30 (m, 10H), 7.40–7.70 (m, 3H), 8.89 (br s, 1H), 9.70 (br s, 1H), 12.19 (br s, 1H). $C_{39}H_{50}N_6O_7$: MS (ES): m/z (%) 715 (100, MH+), 737 (5). HPLC: 100% (ELSD, t_R = 14.1 min).

4.4.9. (*R*)-1-[4-(2,2-Diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]-2-{4-[4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]butanoyl}guanidinium 2,2,2-trifluoroacetate (1l)

The product is a white solid (90 mg, 66%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.25–1.80 (m, 4H), 2.00–2.15 (m, 2H), 2.40–2.50 (m, 2H), 3.15–3.30 (m, 2H), 4.00–4.50 (m, 5H), 4.51 (s, 2H), 5.12 (s, 1H), 6.67 (d, J = 8.38, 2H), 7.00 (d, J = 8.38, 2H), 7.15–7.35 (m, 10H), 7.98 (s, 1H), 8.37 (t, J = 5.69, 1H), 8.40–8.70 (m, 3H), 8.80–10.50 (m, 2H), 11.48 (br s, 1H). $C_{34}H_{40}N_8O_5$: MS (ES): m/z (%) 641 (100, MH+). HPLC: 99% (ELSD, t_R = 12.6 min).

4.4.10. (*R*)-2-{4-[4-(Ammoniomethyl)-1*H*-1,2,3-triazol-1-yl]butanoyl}-1-[4-(2,2-diphenylacetamido)-5-(4-hydroxybenzylamino)-5-oxopentyl]guanidinium 2,2,2-trifluoroacetate (1m)

The product is a white solid (139 mg, 73%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.80 (m, 4H), 2.09 (p, J = 7.20, 2H), 2.45–2.50 (m, 2H), 3.15–3.30 (m, 2H), 4.05–4.20 (m, 4H), 4.25–4.40 (m, 1H), 4.45 (t, J = 7.08, 2H), 5.12 (s, 1H), 6.67 (d, J = 8.45, 2H), 7.00 (d, J = 8.45, 2H), 7.15–7.35 (m, 10H), 8.15 (s, 1H), 8.20–8.35 (br s, 2H), 8.37 (t, J = 5.79, 1H), 8.50 (d, J = 8.02, 1H), 8.55–9.10 (m, 2H), 9.33 (br s, 1H), 12.11 (s, 1H). $C_{34}H_{41}N_9O_4$: MS (LC–MS-I): m/z (%) [t_R = 7.5 min]: 320 (100), 341 (35), 640 (50, MH+). HPLC: 100% (ELSD, t_R = 10.7 min).

4.4.11. (13*R*)-13-(4-Hydroxybenzylcarbamoyl)-3,6,15-trioxo-16,16-diphenyl-1-((2*R*,3*R*,4*S*,5*R*)-3,4,5,6-tetrahydroxytetrahydro-2*H*-pyran-2-yl)-2-oxa-7,9,14-triazahexadecan-8-iminium 2,2,2-trifluoroacetate (1n)

The product is a white solid (35 mg, 76%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.80 (m, 4H), 2.55–2.80 (m, 4H), 3.20–3.90 (m, 5H), 3.90–4.80 (m, 8H), 4.85–5.00 (m, 2H), 5.12 (s, 1H), 5.20–6.50 (m, 1H), 6.67 (d, J = 8.41, 2H), 7.00 (d, J = 8.41, 2H), 7.15–7.35 (m, 10H), 8.38 (t, J = 5.63, 1H), 8.50 (d, J = 8.06, 1H), 8.50–8.70 (br s, 2H), 8.86 (br s, 1H), 9.30 (br s, 1H), 11.49 (br s, 1H). $C_{37}H_{45}N_5O_{11}$: MS (ES): m/z (%) 736 (100, MH+), 758 (20). HPLC: 100% (ELSD, t_R = 12.1 min).

4.4.12. (*R*)-21-Carboxy-4-(2,2-diphenylacetamido)-1-(4-hydroxyphenyl)-3,11,18-trioxo-2,8,10,19-tetraazahenicosan-9-iminium 2,2,2-trifluoroacetate (1o)

1.) Removal of the benzyl-group: The Boc- and ^{t}Bu-protected precursor of compound **1o** (35 mg, 0.04 mmol) was dissolved in 2-propanol (5 mL). Pd/C (10 mg) was added and the mixture was stirred at 15 bar hydrogen pressure in an autoclave. After 2 h TLC control confirmed complete conversion. Pd/C was filtered off over Celite and the solvent was evaporated. The product was obtained quantitative.
2.) The *N*-Boc and *O*-^{t}Bu protected compound was deprotected as described in Section 4.4.

The product is a white solid (30 mg, 78%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.10–1.80 (m, 12H), 2.03 (t, J = 7.28, 2H), 2.30–2.45 (m, 4H), 3.15–3.30 (m, 4H), 4.05–4.25 (m, 2H), 4.25–4.40 (m, 1H), 5.12 (s, 1H), 6.67 (d, J = 8.41, 2H), 7.00 (d, J = 8.38, 2H), 7.15–7.35 (m, 10H), 7.86 (t, 1H), 8.30–8.70 (m, 4H), 8.80–9.00 (m, 1H), 9.20–9.40 (br s, 1H), 11.30–11.40 (br s, 1H). $C_{38}H_{48}N_6O_7$: MS (ES): m/z (%) 351 (11), 701 (100, MH+). HPLC: 99% (ELSD, t_R = 13.7 min).

4.4.13. (*R*)-4-(2,2-Diphenylacetamido)-1-(4-hydroxyphenyl)-9-iminio-3,11,18-trioxo-2,8,10,19-tetraazahenicosan-21-aminium 2,2,2-trifluoroacetate (1p)

The product is a white solid (125 mg, 70%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.10–1.70 (m, 12H), 2.00–2.15 (m, 2H), 2.35–2.45 (m, 2H), 2.75–2.90 (m, 2H), 3.15–3.30 (m, 4H), 4.10–4.20 (m, 2H), 4.25–4.40 (m, 1H), 5.12 (s, 1H), 6.67 (d, J = 8.36, 2H), 7.00 (d, J = 8.36, 2H), 7.15–7.30 (m, 10H), 7.78 (br s, 3H), 8.00 (t, J = 5.52, 1H), 8.38 (t, J = 5.72, 1H), 8.51 (d, J = 8.02, 1H), 8.65–8.80 (br s, 2H), 9.12 (br s, 1H), 9.32 (br s, 1H), 11.73 (br s, 1H). $C_{37}H_{49}N_7O_5$: MS (ES): m/z (%) 336 (100), 672 (25, MH+). HPLC: 97% (ELSD, t_R = 11.2 min).

4.4.14. (*R*)-18-Amino-4-(2,2-diphenylacetamido)-1-(4-hydroxyphenyl)-3,11-dioxo-2,8,10,17-tetraazaoctadecane-9,18-diiminium 2,2,2-trifluoroacetate (1q)

The product is a white solid (93 mg, 71%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, CD_3CN): 1.25–1.40 (m, 2H), 1.45–1.85 (m, 8H), 2.40–2.48 (t, J = 7.27, 2H), 3.00–3.10 (dd, J_1 = 6.68, J_2 = 12.84, 2H), 3.10–3.30 (m, 2H), 4.10–4.25 (m, 2H), 4.30–4.40 (m, 1H), 5.02 (s, 1H), 6.40 (br s, 4H), 6.71 (d, J = 8.50, 2H), 6.90–7.40 (m, 5H), 7.03 (d, J = 8.42, 2H), 7.20–7.35 (m, 10H), 9.45 (br s, 1H), 9.85 (br s, 1H), 12.73 (br s, 1H). ^{13}C NMR (75 MHz, CD_3CN): 23.2, 23.4, 25.0, 27.4, 28.6, 35.9, 40.1, 40.8, 41.8, 52.4, 57.0, 114.8, 126.67, 126.73, 128.14, 128.17, 128.34, 128.40, 129.6, 154.1, 155.7, 171.9, 176.3. $C_{34}H_{44}N_8O_4$: MS (LC–MS-I): m/z (%) [t_R = 7.6 min]: 315 (95), 335 (100), 629 (85, MH+), 675 (30), 743 (5). HPLC: 100% (ELSD, t_R = 11.2 min).

4.4.15. (*R*)-4-(2,2-Diphenylacetamido)-1-(4-hydroxyphenyl)-9-iminio-3,11,14-trioxo-2,8,10,15-tetraazaheptadecan-17-aminium 2,2,2-trifluoroacetate (1s)

The product is a white solid (0.74 g, 68%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.32–1.60 (m, 3H), 1.60–1.76 (m, 1H), 2.46 (t, J = 6.71, 2H), 2.66 (t, J = 6.48, 2H), 2.80–2.88 (m, 2H), 3.18–3.31 (m, 4H), 4.11 (dd, J_1 = 15.02, J_2 = 5.76, 1H), 4.18 (dd, J_1 = 15.05, J_2 = 5.93, 1H), 4.28–4.38 (m, 1H), 5.12 (s, 1H), 6.67 (d, J = 8.53, 2H), 7.00 (d, J = 8.54, 2H), 7.18–7.32 (m, 10H), 7.76 (br s, 3H), 8.14 (t, J = 5.62, 1H), 8.38 (t, J = 5.74, 1H), 8.50 (d, J = 8.06, 1H), 8.71 (br s, 2H), 9.16 (t, non-res., 1H), 9.31 (br s, 1H), 11.88 (s, 1H). $C_{33}H_{41}N_7O_5$: MS (LC–MS-I): m/z (%) [t_R = 7.44 min]: 308.5 (100), 616 (27, MH+). HPLC: 96% (220 nm, t_R = 16.5 min).

4.4.16. (*R*)-1-Amino-16-(4-hydroxybenzylcarbamoyl)-6,9,18-trioxo-19,19-diphenyl-2,5,10,12,17-pentaazanonadecane-1,11-diiminium 2,2,2-trifluoroacetate (1t)

The product is a white solid (35 mg, 49%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, CD_3CN): 1.40–1.80 (m, 4H), 2.49 (t, J = 6.59, 2H), 2.66 (t, J = 6.55, 2H), 3.10–3.30 (m, 6H), 4.10–4.25 (m, 2H), 4.25–4.40 (m, 1H), 5.02 (s, 1H), 6.10–7.50 (m, 10H), 6.70 (d, J = 8.50, 2H), 7.03 (d, J = 8.43, 2H), 7.20–7.35 (m, 10H), 9.39 (br s, 1H), 9.79 (br s, 1H), 12.82 (br s, 1H). $C_{34}H_{43}N_9O_5$: MS (LC–MS-I): m/z (%) [t_R = 7.5 min]: 329 (100), 658 (20, MH+). HPLC: 99% (ELSD, t_R = 10.7 min).

4.4.17. (*R*)-*N*^{1}-[4-(2,2-Diphenylacetamido)-1-(4-hydroxyphenyl)-9-iminio-3,11,14-trioxo-2,8,10,15-tetraazaheptadecan-17-yl]-ethane-1,2-diaminium 2,2,2-trifluoroacetate (1u)

The product is a white solid (62 mg, 39%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.30–1.80 (m, 4H), 2.62–2.70 (t, J = 6.29, 2H), 3.00–3.40 (m, 12H), 4.00–4.20 (m, 2H), 4.25–4.40 (m, 1H), 5.12 (s, 1H), 6.70 (d, J = 8.45, 2H), 7.00 (d, J = 8.45, 2H), 7.15–7.35 (m, 10H), 7.90–9.40 (m, 12H), 12.03 (br s, 1H). $C_{35}H_{46}N_8O_5$: MS (ES): m/z (%) 330 (100), 659 (20, MH+). HPLC: 99% (ELSD, t_R = 9.1 min).

4.5. (*R*)-1-(4-Fluorophenyl)-16-(4-hydroxybenzylcarbamoyl)-1,6,9,18-tetraoxo-19,19-diphenyl-2,5,10,12,17-pentaazanonadecan-11-iminium 2,2,2-trifluoroacetate (1j)

Compound **1s** (24 mg, 28.4 μmol) and NEt_3 (21 μL, 152 μmol) were dissolved in DMF (200 μL) followed by the addition of active ester **37** (4.5 mg, 19 μmol). The reaction was stopped by addition of 10% aq TFA (corresponding to 60 μmol of TFA) after an incubation period of 3.5 h at rt. Purification with preparative HPLC (column: Eurospher-100 C18 (250 × 32 mm, 5 μm), Knauer, Berlin, Germany) and lyophilisation afforded the product as white fluffy solid (10.55 mg, 65%), mp >190 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): 1.31–1.60 (m, 3H), 1.60–1.73 (m, 1H), 2.45 (t, J = 6.60, 2H), 2.64 (t, J = 6.38, 2H), 3.16–3.34 (m, 6H), 4.10 (dd, J_1 = 14.80, J_2 = 5.66, 1H), 4.18 (dd, J_1 = 15.06, J_2 = 5.76, 1H), 4.28–4.38 (m, 1H), 5.12 (s, 1H), 6.67 (d, J = 8.52, 2H), 7.00 (d, J = 8.52, 2H), 7.18–7.33 (m, 12H), 7.86–7.93 (m, 2H), 8.11 (t, J = 5.51, 1H), 8.38 (t, J = 5.78, 1H), 8.47–8.62 (m, 4H), 8.87 (t, non-res., 1H), 9.30 (br s, 1H), 11.44 (s, 1H). $C_{40}H_{44}FN_7O_6$: (LC–MS-I): *m/z* (%) [t_R = 8.69 min]: 738 (100, MH+). HPLC: 98% (220 nm, t_R = 23.7 min).

4.6. (*R*)-4-(2,2-Diphenylacetamido)-1-(4-hydroxyphenyl)-9-iminio-3,11,14-trioxo-*N*-(2-propionamidoethyl)-2,8,10,15-tetraazaheptadecan-17-aminium 2,2,2-trifluoroacetate (1r)

Compound **1u** (35 mg, 35 μmol) and NEt_3 (23 μL, 186 μmol) were dissolved in DMF (200 μL) followed by the addition of active ester **38** (2.4 mg, 14 μmol). The reaction was stopped by addition of 10% aq TFA (corresponding to 80 μmol of TFA) after an incubation period of 3 h at rt. Purification with preparative HPLC (column: Eurospher-100 C18 (250 × 32 mm, 5 μm), Knauer, Berlin, Germany) and lyophilisation afforded the product as white fluffy solid (2.71 mg, 21%), mp >190 °C (decomp.). ^{1}H NMR (600 MHz, DMSO-d_6): 0.99 (t, J = 7.59, 3H), 1.35–1.47 (m, 2H), 1.48–1.56 (m, 1H), 1.62–1.69 (m, 1H), 2.11 (q, J = 7.58, 2H), 2.45 (t, J = 6.73, 2H), 2.49–2.52 (m, 4H) 2.65 (t, J = 6.53, 2H), 2.95–3.01 (m, 2H), 3.19–3.24 (m, 2H), 3.27–3.33 (m, 2H), 4.10 (dd, J_1 = 14.76, J_2 = 5.66, 1H), 4.17 (dd, J_1 = 14.83, J_2 = 6.03, 1H), 4.30–4.35 (m, 1H), 5.11 (s, 1H), 6.66 (d, J = 8.52, 2H), 6.99 (d, J = 8.53, 2H), 7.19–7.31 (m, 10H), 8.00 (t, J = 5.64, 1H), 8.17 (t, J = 5.73, 1H), 8.35 (t, J = 5.82, 1H), 8.44 (br s, 2H), 8.47 (d, J = 8.15, 1H), 8.59 (br s, 2H), 8.93 (t, non-res., 1H), 9.28 (br s, 1H), 11.56 (s, 1H). $C_{38}H_{50}N_8O_6$: (LC–MS-I): *m/z* (%) [t_R = 7.54 min]: 358 (100), 715 (18, MH+). HPLC: 97% (220 nm, t_R = 17.3 min).

4.7. Experimental determination of log *D* values with HPLC

The log *D* values of compounds **1a**, **1d**, **1f**, **1g**, **1i**, **1l**, **1n**, **1p**, **1q** and **1u** were determined by RP-HPLC. A Luna C18 column (3.0 μm, 150 × 2 mm, Phenomenex, Aschaffenburg, Germany) with a flow of 0.30 mL/min was used. An ammonium acetate buffer (10 mM, pH 7.4) was used as solvent A. Solvent B was acetonitrile. The gradient was: 0 min [A/B 95:5], 30 min [A/B 2:98]. A volume of 2 μL of a solution of the analytes, dissolved in DMSO (1 mM), was injected. Column temperature was 25 °C. The compounds were detected with a diode array detector. The log *D* value was calibrated to the retention time (t_R) by running a mixture of six reference compounds and plotting the t_R versus log *D* (Supplementary data).

4.8. Investigation of stability

Decomposition of the N^G-acylated argininamides to **1a** was investigated at neutral pH in the buffer used for radioligand binding[4] (10 mM HEPES, pH 7.4, filtered: 0.45 μm). Incubation was started by addition of 15 μL of a 1 mM solution of the compounds in DMSO to 285 μL of buffer to give a final concentration of 50 μM (in case of **1c** 60 μL of a 0.25 mM solution were added to 240 μL of buffer). After 20 and 90 min a 125 μL aliquot was taken and diluted with a mixture of MeCN, H_2O and 1% aq TFA (for compounds **1c**–**1j**: 5:1:4, 125 μL; for compounds **1k**–**1u**: 1:1:1.33, 125 μL). One hundred microlitres of the resulting solution (pH ≈2) were analysed with analytical 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 0.05% aq TFA (A) and acetonitrile (B) were used as mobile phase. The flow rate was set to 0.80 mL/min and the column temperature to 30 °C. Due to the wide range of log *D* values of the compounds two different gradients were used for HPLC analysis (compounds **1c**–**1j**: 0 min [A/B 75:25], 28 min [A/B 80:20], 30 min [A/B 5:95], 38 min [A/B 5:95]; compounds **1k**–**1u**: 0 min [A/B 85:15], 28 min [A/B 55:45], 32 min [A/B 5:95], 40 min [A/B 5:95]). A five-point calibration curve (0.3, 1.5, 4, 10 and 25 μM) of BIBP 3226 (**1a**) was acquired for quantification of the decomposition product **1a**.

To study the decomposition products of **1j** with LC–MS, **1j** was incubated in 10 mM HEPES buffer as described above, but at a concentration of 30 μM (30 μL of a 0.5 mM solution of **1j** in MeCN/0.05% aq TFA (20:80) to 470 μL of buffer). After 20 and 90 min a 125 μL aliquot was taken and diluted with a mixture of MeCN, H_2O and 1% aq TFA (1:1:1.33, 125 μL). Ten microlitres of the resulting solution were injected into the LC–MS system and analysed with LC–MS method II (cf. Section 4.1) (source type: ESI (capillary temperature: 250 °C, spray voltage: 4.0 kV, sheath and auxiliary gas: on).

4.9. Radioligand competition binding assay

Radioligand competition experiments at SK-N-MC neuroblastoma cells using the radioligand [^{3}H]UR-MK114 (1.5 nM) were performed as described elsewhere.[4]

4.10. Fura-2 assay on HEL cells

The Fura assay was performed with HEL cells as previously described using a Perkin–Elmer LS50 B spectrofluorimeter (Perkin–Elmer, Überlingen, Germany).[19]

4.11. Data processing

Data from radioligand competition experiments were analyzed by four parameter sigmoidal fits (SigmaPlot 9.0, Systat Software). IC_{50} values from radioligand competition studies were converted to K_i values according to the Cheng–Prusoff equation[34] using the respective K_D value of the radioligand. Three data points (between 20% and 80% inhibition, from fura-2 assays) served for the calculation of IC_{50} values after logit-log transformation. IC_{50} values were converted to K_b values according to the Cheng–Prusoff equation[33] using an EC_{50} value of 1.8 nM for pNPY (mean value from four independently determined concentration–effect curves on HEL cells).

Acknowledgement

This work was supported by the Graduate Training Program (Graduiertenkolleg) GRK 760, 'Medicinal Chemistry: Molecular Recognition—Ligand-Receptor Interactions', of the Deutsche Forschungsgemeinschaft.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.028.

References and notes

1. Keller, M.; Teng, S.; Bernhardt, G.; Buschauer, A. *ChemMedChem* **2009**, *4*, 1733.
2. Weiss, S.; Keller, M.; Bernhardt, G.; Buschauer, A.; Konig, B. *Bioorg. Med. Chem.* **2008**, *16*, 9858.
3. Hutzler, C.; Kracht, J.; Mayer, M.; Graichen, F.; Bauer, B.; Schreiber, E.; Bollwein, S.; Bernhardt, G.; Dove, S.; Fricker, G.; Buschauer, A. *Arch. Pharm. (Weinheim)* **2001**, *334*, 17.
4. Keller, M.; Pop, N.; Hutzler, C.; Beck-Sickinger, A. G.; Bernhardt, G.; Buschauer, A. *J. Med. Chem.* **2008**, *51*, 8168.
5. Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. *ChemMedChem* **2009**, *4*, 225.
6. Schneider, E.; Keller, M.; Brennauer, A.; Hoefelschweiger, B. K.; Gross, D.; Wolfbeis, O. S.; Bernhardt, G.; Buschauer, A. *Chembiochem* **2007**, *8*, 1981.
7. Brennauer, A.; Dove, S.; Buschauer, A. Structure–Activity Relationships of Nonpeptide Neuropeptide Y Receptor Antagonists. In *Handbook of Experimental Pharmacology*, Michel, M.C., Ed.; 2004; Vol. 162.
8. Dove, S.; Elz, S.; Seifert, R.; Buschauer, A. *Mini-Rev. Med. Chem.* **2004**, *4*, 941.
9. Ghorai, P.; Kraus, A.; Keller, M.; Gotte, C.; Igel, P.; Schneider, E.; Schnell, D.; Bernhardt, G.; Dove, S.; Zabel, M.; Elz, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2008**, *51*, 7193.
10. Igel, P.; Schneider, E.; Schnell, D.; Elz, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 2623.
11. Igel, P.; Geyer, R.; Strasser, A.; Dove, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 6297.
12. Kraus, A.; Ghorai, P.; Birnkammer, T.; Schnell, D.; Elz, S.; Seifert, R.; Dove, S.; Bernhardt, G.; Buschauer, A. *ChemMedChem* **2009**, *4*, 232.
13. Brennauer, A. Doctoral thesis, Thesis 2006.
14. Schneider, E.; Mayer, M.; Ziemek, R.; Li, L.; Hutzler, C.; Bernhardt, G.; Buschauer, A. *Chembiochem* **2006**, *7*, 1400.
15. Michel, M. C.; Beck-Sickinger, A.; Cox, H.; Doods, H. N.; Herzog, H.; Larhammar, D.; Quirion, R.; Schwartz, T.; Westfall, T. *Pharmacol. Rev.* **1998**, *50*, 143.
16. Rudolf, K.; Eberlein, W.; Engel, W.; Wieland, H. A.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Beck-Sickinger, A. G.; Doods, H. N. *Eur. J. Pharmacol.* **1994**, *271*, R11.
17. Brennauer, A.; Keller, M.; Freund, M.; Bernhardt, G.; Buschauer, A. *Tetrahedron Lett.* **2007**, *48*, 6996.
18. Kerns, E. H.; Di, L.; Petusky, S.; Kleintop, T.; Huryn, D.; McConnell, O.; Carter, G. *J. Chromatogr., B* **2003**, *791*, 381.
19. Müller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A. *Arch. Pharm. (Weinheim)* **1997**, *330*, 333.
20. Knieps, S.; Dove, S.; Michel, M. C.; Rottmeier, K.; Werner, W.; Bernhardt, G.; Buschauer, A. *Pharm. Pharmacol. Lett.* **1996**, *6*, 27.
21. Beck-Sickinger, A. G.; Wieland, H. A.; Wittneben, H.; Willim, K. D.; Rudolf, K.; Jung, G. *Eur. J. Biochem.* **1994**, *225*, 947.
22. Khoukhi, N.; Vaultier, M.; Carrie, R. *Tetrahedron* **1987**, *43*, 1811.
23. Fan, X.; Potluri, V. K.; McLeod, M. C.; Wang, Y.; Liu, J.; Enick, R. M.; Hamilton, A. D.; Roberts, C. B.; Johnson, J. K.; Beckman, E. J. *J. Am. Chem. Soc.* **2005**, *127*, 11754.
24. Bartoli, G.; Bosco, M.; Locatelli, M.; Marcantoni, E.; Melchiorre, P.; Sambri, L. *Org. Lett.* **2005**, *7*, 427.
25. Qiao, C.; Jeon, H.-B.; Sayre, L. M. *J. Am. Chem. Soc.* **2004**, *126*, 8038.
26. Kartha, K. P. R. *Tetrahedron Lett.* **1986**, *27*, 3415.
27. Bartalucci, G.; Bianchini, R.; Catelani, G.; D'Andrea, F.; Guazzelli, L. *Eur. J. Org. Chem.* **2007**, 588.
28. Drake, B.; Patek, M.; Lebl, M. *Synthesis* **1994**, 579.
29. Carmignani, M.; Volpe, A. R.; Botta, B.; Espinal, R.; De Bonnevaux, S. C.; De Luca, C.; Botta, M.; Corelli, F.; Tafi, A.; Sacco, R.; Delle Monache, G. *J. Med. Chem.* **2001**, *44*, 2950.
30. Moura, C.; Vitor, R. F.; Maria, L.; Paulo, A.; Santos, I. C.; Santos, I. *Dalton Trans.* **2006**, 5630.
31. New, O. M.; Dolphin, D. *Eur. J. Org. Chem.* **2009**, 2675.
32. English, A. R.; Girard, D.; Jasys, V. J.; Martingano, R. J.; Kellogg, M. S. *J. Med. Chem.* **1990**, *33*, 344.
33. Vollmann, H.; Becker, H.; Corell, M.; Streeck, H.; Langbein, G. *Justus Liebigs Ann. Chem.* **1937**, *531*, 1.
34. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.