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Discovery and characterization of biased allosteric agonists of the chemokine receptor CXCR3

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ABSTRACT

In this work we report a design, synthesis and detailed functional characterization of unique strongly biased allosteric agonists of CXCR3 that contain tetrahydroisoquinoline carboxamide cores. The compound **11** (FAUC1036) is the first strongly biased allosteric agonist of CXCR3 that selectively induces weak chemotaxis, leads to receptor internalization and the β-arrestin 2 recruitment with potency comparable to that of the chemokine CXCL11 without any activation of G proteins. A subtle structural change (addition of a methoxy group, **14** (FAUC1104)) led to a contrasting biased allosteric partial agonist that activated solely G proteins, induced chemotaxis but failed to induce receptor internalization or β-arrestin 2 recruitment. Concomitant structure-activity relationship studies indicated very steep structure-activity relationships, which steer the ligand bias between the β-arrestin 2 and G protein pathway. Overall, the information presented provides a powerful platform for further development and rational design of strongly biased allosteric agonists of CXCR3.

Introduction

Biased signaling is a now well recognized phenomenon observed in the G protein coupled receptors (GPCRs) that describes the ability of different ligands acting at the same GPCR to stabilize distinct receptor conformations that, in turn, are linked to different functional outcomes.[1–3] In the case of the human chemokine receptor CXCR3, which binds three endogenous chemokines CXCL9, CXCL10 and CXCL11, biased signaling is used by nature to fine tune the responses of $CXCR3^+$ cells upon physiological insult.[4] CXCR3 expression is rapidly induced on naïve T cells following activation, and preferentially remains highly expressed on type1 helper (Th1) $CD4^+$ T cells, effector $CD8^+$ T cells and innate-type lymphocytes, such as natural killer (NK) and natural killer T (NKT) cells.[5,6] Depending on the endogenous chemokine bound, the activation of CXCR3 leads to distinct functional outcomes, which considerably influence the course of inflammation and healing processes.[5,7] The opportunity to mimic this mechanism by small molecule allosteric ligands has remained largely unexploited, even though CXCR3 has been deemed as a promising therapeutic target in pathologies like autoimmune diseases (e.g., rheumatoid arthritis, multiple sclerosis), cancer and metastasis.[8–12] To inhibit the inflammatory response driven by the $CXCR3^+$ cells, published research was focused solely on the development of small-molecules with inhibitory effects on the CXCR3, which ultimately failed to deliver therapeutics.[13–15]A single study used metal-ion site engineering to probe the molecular mechanism of CXCR3 activation and identified high-potency allosteric agonists based on complexes containing Cu or Zn and phenantroline or bipyridine.[16] Beside this, the development of small-molecule agonists of CXCR3 was largely neglected and only a few agonists were reported (Figure 1)[17–19], despite rising indications that CXCR3 agonists may have significant therapeutic potential in wound healing[20] and anti-inflammatory therapy.[21,22]

The general focus of our group is the understanding of the structure-activity relationships (SAR) of allosteric modulators of CXCR3 with probe-dependent behavior and biased signaling.[23–25] The unique nature of allosteric ligands (reflected in permissivity, saturability of effect, probe dependence and biased signaling) offers great potential to extend therapeutic effects compared to orthosteric ligands,[2,4] and is still a poorly explored opportunity. In this work we focused our research on the allosteric agonists of CXCR3.

The allosteric modulators of CXCR3 based on the tetrahydroisoquinoline carboxamide core were previously reported as allosteric agonists of CXCR3 (Figure 1) with no noticeable ligand-directed signaling.[17,19] Our own explorations of this promising scaffold resulted in **11** (FAUC1036), a strongly biased allosteric agonists of CXCR3 (Figure 2 and 3). **11** selectively induces chemotaxis and receptor internalization, and induces β-arrestin 2 recruitment without any stimulation of G proteins. During subsequent structure-activity studies and detailed pharmacological profiling of these novel compounds we unambiguously determined crucial structural elements that drive biased signaling of these unique allosteric agonists. We discovered a molecular switch that steers ligand-induced biased signaling between the β-arrestin 2 and G protein pathway as shown in **14** (FAUC1104) (Figure 3) that activates G proteins and induces chemotaxis, but cannot induce receptor internalization and β-arrestin 2 recruitment.

Results and Discussion

Based on our initial hit, the strongly biased allosteric agonist **11**, we synthesized an enantiomerically and epimerically pure series (Supplemental Figure S4) of derivatives focusing on variations of the northern side chain (Figure 2). We combined this approach with detailed pharmacological characterization of novel compounds that allowed us to gain novel

insight into the structure-activity relationships for the fine control of allosteric modulator potency, cooperativity and biased signaling at CXCR3.

Synthesis of allosteric ligands

The preparation of the **11** began with an acylation step of diphenyl ethylamine using Boc-Lys(Z)-OH in presence of EDC hydrochloride (compound **1**), followed by hydrogenolysis to afford the primary amine **2**. Then, Cbz protection of the commercially available (*S*)-2-((benzyloxy)carbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid using benzyl chloroformate in basic medium[26] to give compound **3** followed by an amide coupling step using **2** resulted in the formation of compound **4**. Hydrogenolysis yielded compound **5**, a precursor ready to be coupled to the northern side chain in one step (Scheme 1).

For the variation of the northern side chain several non-aromatic heterocyclic acids **6-8** were synthesized by acylating piperidine, morpholine and *N*-methyl piperazine, respectively with succinic anhydride at room temperature.[27,28] The aromatic heterocyclic acids **9, 10** were obtained by a Friedel Crafts acylation of furan and thiophene with succinic anhydride as well[29,30] (Scheme 2). The remaining carboxylic acids were commercially available. All acids were coupled to **5** using EDC hydrochloride and DIPEA to afford the final compounds **11 - 24** (Scheme 3). Compound **27** was produced by acylating of **5** with succinic anhydride. The Boc group of compounds **11**, **24** and **5** was removed with TFA to yield the protonated compounds **25**, **26**, **28** respectively.

Furthermore, the *(S)*-configured-lysine moiety of **11** and **25** was replaced with the *(R)*-lysine resulting in the compounds **34** and **35**. The synthetic strategy of **29-35** was identical to that of **11** (supporting information, Supplemental Scheme 1).

All compounds mentioned above are free of contaminants, which could have arisen due to racemization during the activation process of the amino acids. This has been demonstrated by additionally recording spectra in $CDCl_3$, since they unambiguously allowed to discriminate key proton resonances of both the epimers **11** (*S,S*) and **34** (*S,R*) (see Supporting Figure S4). In neither recorded sample of these two compounds, the resonances of the corresponding epimer could be detected. This allows the conclusion, that also the precursor **5** leading to the other target compounds **12-28** does not contain the corresponding epimer either. Due to the missing tendency of enantiomerization during the peptide coupling process, we are also able to exclude the potential formation of the (*R,R*) enantiomer and the (*R,S*) epimer (which should give the same set of signals like the (*S,R*) isomer **34**) of target compound **11**.

To enable appropriate comparison of reference compound **46**[17] **(**VUF10661, see Figure 1) with our novel ligands, we developed analogues by inverting the lysine's center of chirality to give the epimer **47**. Furthermore, we decided to test the Boc-protected intermediate **44** and **45**, in order to investigate the influence of the bulky, uncharged side chains compared to the protonated aminoalkyl side chains of the compounds **46** and **47**. The positively charged amine groups were reported to be crucial for receptor activation.[18,19] For the synthesis of compound **46** and its analogues, we followed a strategy similar to that eported in Scheme 1 (supporting information, Supplemental Scheme 2), which gave significantly higher yields than those found in the literature.[19]

Pharmacological characterization

The binding of endogenous agonists CXCL11, CXCL10 and CXCL9 to CXCR3 results in the activation of $G\alpha_i$ proteins, recruitment of β-arrestins, receptor internalization and chemotaxis. To elucidate the pharmacological properties of derivatives of **11**, we focused on the ability of derivatives to modulate G protein activation and β-arrestin 2 recruitment. For

the most prominent compounds **11** and **14** their capacity to induce CXCR3 receptor internalization and chemotaxis was investigated in detail. Prior to functional characterization the potential cytotoxicity of derivatives was determined with ApoTox-Glo Triplex assay (Promega), which easily assesses viability, cytotoxicity and apoptosis events in the same cell-based assay. None of derivatives exhibited nonspecific effects on the cells (supplemental information, Supplementary Figure S1). The activation of G proteins was explored in terms of CXCR3 mediated [^{35}S]GTPγS incorporation.

The activation of G proteins was monitored as the incorporation of [^{35}S]GTPγS and thus increase in the incorporated radioactivity. The potency and efficacy of our novel compounds was first compared to the reference compound **46**. The compound **46** produced a dose-dependent increase in the [^{35}S]GTPγS incorporation with a potency of 0.6 μM (pEC_{50}= 6.2 ± 0.1), which is in accordance with the published data (Table 1).[19] The epimer of **46** compound **47** as well as the Boc protected derivatives **44** and **45** showed no significant changes in potency and efficacy. Although it was reported that the charge at the amine on the side chain of the lysine moiety was crucial for the biological activity of **46**,[18,19] we clearly demonstrate that this charge is fully dispensable for the activation of G proteins by our allosteric CXCR3 agonists.

Strikingly, **11** failed to induce CXCR3 mediated G protein activation, just as its unprotected form **25** and its epimer **34** (Table 2). The epimer of **25** compound **35** showed some weak partial agonism at pEC_{50} of 8.01±0.31. Compound **22**, where the keto group of the benzoyl moiety was removed, showed very low potency (pEC_{50} = 5.07±0.50) as well. To determine the influence of substitution pattern using polar groups at the *para* position of the phenyl ring on the northern side chain, fluorine (**13**), methoxy (**14**) and methyl sulfonyl (**15**) groups were introduced. This modification resulted in an increase of the intrinsic agonist properties of compounds. The introduction of methoxy group (**14**) caused the most significant

increase in the efficacy (50%; the pEC_{50} value of 5.22±0.31). An increase of the electron density near the receptor by an addition of a second methoxy group at the *meta* position (**21**) caused a surprising reduction of the efficacy (18%), which indicated that the activity is not dependent on the electron density of the phenyl ring. Therefore, we removed the aromaticity by replacing the phenyl ring of the northern side chain with a piperidine moiety (**16**), however no reactivity was observed. Knowing that the polar substitution of the phenyl ring increased the reactivity of the ligands in the G protein activation pathway, we introduced a polar morpholine (**17**) and a charged *N*-methyl piperazine moiety (**18**), in order to improve the reactivity of the compound **16**. The results showed that non-aromatic heterocycles have no effect in the [^{35}S]GTPγS assay. Similar results were obtained for the aromatic heterocycles **19** and **20**, which might indicate that the length of the northern side chain is too short for creating a possible hydrogen bond/s with the receptor compared to **14**. We therefore generated analogues **5**, **23** and **24** in which we varied the length of a spacer in the northern side chain. Compound **5** showed the most interesting results, where the efficacy in the [^{35}S]GTPγS incorporation increased to 34% with the EC_{50} value of 2.2 μM (pEC_{50} = 5.7 ± 0.34) indicating the importance of the specific side chain for keeping the G protein inactive. Finally, Boc-free compounds **26** and **28** with modified spacer of the northern side chain showed no significant changes compared to their Boc analogues **24** and **5**, respectively, showing that the Boc group or the positive charge are not crucial for the G protein activation.

Importantly, all novel ligands that showed no intrinsic agonist activity were tested for the ability to inhibit the CXCL11 or CXCL10 mediated activation of G proteins. None of the ligands were able to inhibit the activation of CXCR3 by its endogenous chemokine ligand.

The ability of our novel ligands to induce CXCR3-mediated β-arrestin 2 recruitment was investigated in the assay system PathHunter (DiscoveRx), which detects the recruitment of β-arrestin 2 to the activated receptor using β-galactosidase (β-gal) enzyme fragment complementation. We used two different ProLink tags, ProLink1 (PK1) and ProLink2 (PK2), of which PK2 has higher affinity for the enzyme acceptor to detect weak protein-protein interactions. The CXCR3-PK2 construct is readily able to detect the differences in the propensity of CXCL11 and CXCL10 to induce β-arrestin 2 recruitment at CXCR3 (supplemental information, Supplementary Figure S2). As reported, the reference compound **46** showed the pEC_{50} value 5.89 ± 0.07 and nearly 40% higher efficacy than CXCL11.[19] Contrary to the G protein activation, the enantiomer of **46** **47** and the Boc protected derivatives **44** and **45** had a reduced ability to induce recruitment of β-arrestin 2 to CXCR3 (Table 3). The most striking difference was observed for the Boc protected enantiomer of **46**, compound **45**, which showed the reduced efficacy of about 36% compared to **46**. The presence of the positive charge at the amine on the southern side chain seems to significantly contribute to the activation of the β-arrestin 2 pathway.

11 emerged as a strongly biased allosteric partial agonist as it activated the recruitment of β-arrestin 2 to CXCR3 with a pEC_{50} of 6.64 ± 0.38 and the efficacy of 30% (measured with PK1 construct, Table 4), whereas no G protein activation could be observed for this compound. Every substitution on the phenyl ring of the northern side chain, fluorine (**13**), methoxy (**14**), sulfone (**15**), dimethoxy (**21**) groups resulted in complete loss of intrinsic agonistic properties in the β-arrestin 2 recruitment assay, results that suggest phenyl ring polar substitutions enhance biased agonism by preferentially activating G protein over recruitment of β-arrestin 2. Replacement of the benzoyl group with an acetyl group (compound **12**) or increase of the spacer length (compound **27**) behaved as strongly biased

allosteric agonists with efficacies and potencies comparable to **11** (Table 4). For the compounds **22** and **23**, where the keto group of the benzoyl moiety was removed, a significant loss of efficacy is observed indicating the importance of that group with respect to β-arrestin 2 recruitment. The *S,R*-epimer **34** reached the levels of full agonism in assays using PK2 construct. The charged analogues **25**, **28** and **35** showed a decrease of efficacy. Compound **5**, which is devoid of the entire northern fragment, retained its ability to recruit β-arrestin 2 to CXCR3 (the pEC_{50} value 6.50±0.17, efficacy 31%), and showed no bias between the investigated pathways. Finally, the rest of the compounds, **16**-**20** and **27**, proved to have no agonistic effect in the given assays.

GPCRs are generally rapidly desensitized, internalized and degraded upon agonist stimulation.[31–33] The β-arrestin proteins are generally involved in the internalization of GPCRs.[34] Although it has been shown that β-arrestin is involved in CXCR3 internalization to some extent, it is not the sole determinant of this process, and the exact mechanism (both β-arrestin dependent and independent) of CXCR3 internalization is still unclear.[32,33,35] For the chemokine receptors it is known that also a β-arrestin independent type of internalization plays an important role.[36] E.g., for the viral chemokine receptor US28 it was reported that the receptor endocytosis does not require β-arrestins and is dependent on the clathrin-mediated pathway.[37] For the chemokine receptor CCR5 it was reported that caveolae are involved in receptor internalization.[38] Overall, it seems that in chemokine receptors total receptor internalization is the sum of β-arrestin dependent and –independent events. To investigate the effect of **11** (β-arrestin 2 biased allosteric agonist) and **14** (G protein biased allosteric agonist) on internalization, we employed scanning laser confocal microscopy and an ELISA-based assay. The HEK293T cells were transiently transfected by CXCR3-eGFP and either left untreated or stimulated with CXCL11 and **11** or **14** for one hour. As evident in Figure 4A, the treatment with either CXCL11 or **11** induced the CXCR3 internalization. Untreated cells also

show minor internalization of CXCR3. This observation is in accordance with the published observation that CXCR3 is constitutively internalized to some extent.[39] **14** did not induce receptor internalization beyond the internalization observed in the untreated cells. The quantification of receptor internalization upon the treatment with the endogenous agonists and with either β-arrestin 2 biased **11** or G-protein biased **14** was performed with an ELISA based assay. The HEK293T cells transiently expressing FLAG-CXCR3 were stimulated with compounds and chemokines for one hour, washed, fixed and the amount of FLAG-CXCR3 present on the surface was detected by anti-FLAG antibody. As evident from Figure 4B, only CXCL11 and **11**, the β-arrestin 2 biased allosteric agonist, induced receptor internalization. These experiments also demonstrated the unique value of our ligands to describe signaling events because they remove one of the signals from an otherwise normal activation event.

The ligands **11** and **14** clearly displayed multiple dimensions of biased signaling with **11** stimulation the β-arrestin 2 recruitment and receptor internalization, and **14** the activation of G proteins only. To estimate potential cooperativity between those ligands and endogenous chemokine ligand CXCL11, we performed radioligand binding assay using [^{125}I]CXCL11 (Figure 5A and B). The data showed that neither **11** nor **14** were able to suppress the binding of CXCL11 to CXCR3 (Figure 5A). Our ligands thus bind to an allosteric binding site of CXCR3 and do not exert any cooperativity toward CXCL11. This observation was further confirmed by the experiment in which the fixed concentration (1 μM) of **11** and **14** was used and increasing concentrations of CXCL11. It is evident that the presence of either **11** or **14** has no influence on the binding of CXCL11 (Figure 5B). Our ligands are thus allosteric agonists. Similar observation was previously reported also for **46**.[19] The lack of cooperativity of between the **11** and CXCL11 was observed also in the β-arrestin 2 recruitment assay (Figure 5C). We decided to determine the potential for CXCL11-mediated beta arrestin recruitment to CXCR3 receptors already responding to **11**, and thus extended the ligand

response time. During this experiment we observed some minor reduction in the basal β-arrestin 2 recruitment. The data in Figure 5C are normalized in a manner that makes the basal β-arrestin 2 recruitment visible. The maximal stimulation of 100% is defined by the maximal response exerted by CXCL11. Because our ligand do not show any toxicity (Supplemental Figure 1), we assume that this could be a result of a long treatment period (of four hours) in the combination with high basal β-arrestin 2 recruitment to CXCR3 that results in the receptor internalization and thus causes desensitation, which is defined as the fading of a receptor response to persistent agonist stimuli.[40] The demonstrated internalization caused by **11** (Figure 4) would inhibit constitutive CXCR3 activity, but being allosteric, would not affect the potency of CXCL11 on those receptors still available on the cell surface.

Chemotaxis represents the crucial and defining functional response induced by the activation of chemokine receptors.[12,41–43] Signaling through $G\alpha_i$ has been shown to activate CXCR3 induced migration through multiple pathways, including p38 and PI3K in human epithelial airway cells[44] and PI3K and PTEN in human neutrophils[45]. No direct studies on CXCR3 induced β-arrestin mediated migration has been published, but it is possible that CXCR3 chemokines convey some of their migratory stimulation through β-arrestin induced signaling, as reported for CXCL11 at the decoy receptor ACKR3/CXCR7 and for angiotensin II at the angiotensin II type 1a receptor.[46,47] To investigate the influence of **11** (β-arrestin 2 biased allosteric agonist) and **14** (G protein biased allosteric agonist) on CXCR3 induced migration, we used these compounds alongside CXCL11 in a standard transwell assay with HEK293 cells transiently transfected with CXCR3. When applied to the source reservoir, both compounds on their own induced migration approximately 0.25 fold compared to buffer control, which is less than the maximal effect observed with CXCL11 (0.7 fold), but still significant, indicating that both compounds positively influence migration (Figure 6A). This means that in CXCR3, neither G protein activation, nor β-arrestin recruitment, can adequately

account for the final functional chemotaxis response. To test whether **11** and **14** influence CXCL11 induced migration, we pre-incubated the cells with these compounds for 15 minutes and then set up the migration assay in the presence of either compound in both the top chamber (sink) containing the cells and in the lower chamber (source) containing CXCL11 as chemoattractant. We observed no antagonizing effect of these compounds on CXCL11 induced migration (Figure 6B). Previously reported CXCR3 agonists (Figure 1) were shown to induce migration in the absence of endogenous chemokines.[17,19,22] Only **48** (PS372424) was tested for its influence on CXCL11 induced migration and it was, interestingly, found to antagonize CXCL11 induced migration in activated T-cells.[22]

Overall, detailed pharmacological characterization of the most prominent derivatives **11** and **14** demonstrated the multiple dimensions of biased agonism induced by these compounds and importantly contributed to the dissection of the CXCR3 signaling involved in receptor internalization and chemotaxis. With the aid of **11**, the β-arrestin 2 biased allosteric agonist, and **14**, the G protein biased allosteric agonist, we: (1) identified molecular switches which steer the ligand bias between the β-arrestin 2 and G protein pathway (Figure 7); (2) demonstrated that the CXCR3 internalization is largely β-arrestin 2 dependent or at least G protein independent; and, (3) showed in CXCR3, neither G protein activation, nor β-arrestin recruitment, can adequately account for the final functional chemotaxis response.

Conclusions

Selective regulation of CXCR3 signaling by an appropriate allosteric ligand could be an attractive therapeutic strategy in inflammatory diseases. However, the complex pharmacology of the CXCR3 signaling system poses a nontrivial challenge for the design of biased ligands that selectively activate desired pathways. In this work we report unique strongly biased allosteric agonists of CXCR3 that contain a tetrahydroisoquinoline core. **11**

is the first strongly biased allosteric agonist of CXCR3 that exclusively stimulates receptor internalization and β-arrestin 2 recruitment with a potency comparable to the endogenous chemokine CXCL11 and an efficacy of 66% (using CXCR3-PK2) without any activation of G proteins (Figure 3). An additional methoxy group led to the strongly biased allosteric agonist **14** that activated solely G proteins (a pEC_{50} value 5.22, efficacy 50%). Concomitant structure-activity studies identified molecular switches which steer the ligand bias between the β-arrestin 2 and G protein pathway (Figure 7). Detailed analysis of the influence of **11** and **14** on receptor internalization and chemotaxis showed that **11** induces β-arrestin 2 dependent receptor internalization and chemotaxis; **14** induces only G protein dependent chemotaxis.

With good reason, it is broadly acknowledged that biased ligands represent an opportunity to induce precisely defined GPCR signaling, without casually activating irrelevant pathways, which many speculate to be a major source of side effects in current medical drugs. For practical reasons, the most effective benchmark assays to characterize receptor signaling provide very narrow information, but it is vital to always interpret those as a fragment of a larger range of signaling pathways. One of the most important lessons of **11** and **14** is that β-arrestin 2 and large $G\alpha_i$ protein subunit signaling are dispensable to produce an important functional response - chemotaxis. It is possible that a cross-talk occurs between β-arrestin 2 and $G\alpha_i$ signaling as a late pathway event, perhaps as a backup mechanism, which would make it very difficult to produce true biased signaling. However, it seems more likely that another, distinct, pathway has a stronger connection to chemotaxis, such as $G\alpha_{12/13}$, which has already been connected to T cell adhesion[48]. **11** and **14** are rare examples of fully biased synthetic agonists, and will be valuable tools to dissect and describe CXCR3 responses in a variety of physiologically applicable contexts.

Experimental section

Chemistry

General methods

All reactions requiring anhydrous conditions were carried out under nitrogen and the glassware were dried in flame temperature under vaccum before use. All reactions were monitored by TLC using Kieselgel 60 F_{254} plates. Visualization of the reaction components was achieved using UV fluorescence (254 nm) and $KMnO_4$ stain. Silica gel chromatography was carried out on Kieselgel 60. The yields are reported after purification. ^{1}H, ^{13}C NMR spectra were recorded in deuterated solvents and chemical shifts (δ) are quoted in parts per million (ppm) calibrated to TMS (^{1}H and ^{13}C δ = 0). Coupling constants (*J*) are measured in Hertz (Hz). The following abbreviations are used to describe multiplicities: s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, q = quartet, br s = broad singlet, m = multiplet. Electron-ionization (EI) MS measurements were performed on a JEOL JMS-GC Mate II spectrometer, capable of 5.000 FWHM. Detection was in positive ion mode and source voltage of 70eV. The machine was calibrated prior to every experiment via direct infusion of the low concentration tuning mixture, which provided an m/z range of singly charged peaks. Electrospray-ionization MS (ESI-MS) measurements were performed on a UHR-TOF Bruker Daltonik (Bremen, Germany) maXis plus 5G, an ESI-ToF MS capable of resolution of at least 60.000 FWHM. Detection was in positive ion mode, the source voltage was 2.8 kV. The flow rates were 180 μL/hour. The drying gas (N_2), to aid solvent removal, was held at 180 °C and the spray gas was held at 20 °C. The machine was calibrated prior to every experiment via direct infusion of the Agilent ESI-TOF low concentration tuning mixture, which provided an m/z range of singly charged peaks up to 2700 Da in both ion modes. Optical rotation was measured using a JASCO P-2000 polarimeter, with Na lamp at

589 nm. Purity and identity were assessed by analytical RP-HPLC (Agilent 1100 analytical series, column: Zorbax Eclipse XDB-C8 , 4.6 × 150 mm, 5 μm, flow rate: 0.5 ml/min, detection wavelength: 254 nm) coupled to a Bruker Esquire 2000 mass detector equipped with an ESI-ioniser. Purities of the products were assessed from HPLC-MS using MeOH / H_2O 0.1% HCOOH as the solvent system and employing the following gradient systems:

LC-MS:

Gradient A: 0-3 min: 10% MeOH, 3-18 min: 10-100% MeOH, 18-24 min: 100% MeOH, 24-30 min: 100-10% MeOH.

Gradient B: 0-2 min: 10% MeOH, 2-10 min: 10-100% MeOH, 10-14 min: 100% MeOH, 14-16 min: 100-10% MeOH, 16-18 min: 10% MeOH.

The purity of final products was ≥95%.

NMR performance at high temperature.

NMR analysis at high temperature was performed for compounds with multiple isomers, in order to avoid as much as possible multiple peaks due to isomerism. (or similar).

Preparative HPLC

Preparative RP-HPLC was performed using Agilent 1100 preparative series. Column: Nucleodur C18 HTec, 32 × 250 mm, 5 μm particles, flow rate 32 mL/min, detection wavelengths: 210, 254 nm. The solvent system, that was used for the preparative HPLC, was acetonitrile (ACN), water with 0.1% trifluoroacetic acid (TFA). The methods differ only in gradient.

Method A

20-80% ACN in 0-20 min, 80-95% ACN in 20-22 min, 95-95% ACN in 22-25 min, 95-20% ACN in 25-30 min.

Method B

30-70% ACN in 0-20 min, 70-95% ACN in 20-22 min, 95-95% ACN in 22-25 min, 95-30% ACN in 25-30 min.

Method C

20-70% ACN in 0-20 min, 70-95% ACN in 20-22 min, 95-95% ACN in 22-25 min, 95-20% ACN in 25-30 min.

Method D

40-90% ACN in 0-20 min, 90-95% ACN in 20-22 min, 95-95% ACN in 22-25 min, 95-40% ACN in 25-30 min.

Method E

20-60% ACN in 0-20 min, 60-95% ACN in 20-22 min, 95-95% ACN in 22-25 min, 95-20% ACN in 25-30 min.

General procedure 1 (GP1) for amide couplings: Representative experimental procedure for the amide formation using primary amines.

To solution of acid (1 eq.) in anhydrous DCM was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) hydrochloride (1.1 eq.) at 0 °C and stirred for 1h. The primary amine (1.1 eq.) was added to the reaction mixture, and then it was warmed up to room temperature and stirred for 14-18 h. A saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with DCM (3 times). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude mixture

was purified by flash column chromatography to afford the compounds **1**, **4**, **30**, **32**, **36**, **37**, **40**, **41**.

General procedure 2 (GP2) for amide couplings: Representative experimental procedure for the amide formation using secondary amines.

To a solution of acid (1.1 eq.) in anhydrous DCM were added *N'*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) hydrochloride (1.1 eq.) and *N*,*N*-diisopropylethylamine (1.5 eq.) at 0 °C. The reaction mixture stirred for 30 min. The secondary amine (1 eq) was added, then the reaction mixture warmed up to room temperature and stirred for 16-20 h. A saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with DCM (3 times). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude mixture was purified by flash column chromatography to afford the compounds **11**, **13-22**, **24**, **34**, **35**, **44**, **45**.

General procedure 3 (GP3) for hydrogenation: Representative experimental procedure for the Cbz deprotection.

The protected amine (1 eq.) was dissolved in anhydrous methanol and the solution was transferred to a dry schlenk flask containg Pd/C (0.2 eq.) under counter current at room temperature. The reaction mixture stirred for 6 h under H_2 atmosphere at room temperature. Then the catalyst was removed by filtration (celite pad) and the solvent was evaporated *in vacuo*. To afford the final compounds **2**, **5**, **31**, **33**, **28**, **38**, **39**, **42**, **43**. No further purification was required, however samples were purified by flash column chromatography for their analysis.

General procedure 4 (GP4) for Boc deprotection: Representative experimental procedure for the TFA deprotection.

The Boc protected amine was added in a solution of DCM/TFA (4:1). The reaction mixture stirred for 3 h at room temperature. Then the reaction mixture evaporated *in vacuo*. The precipitate was neutralized with a saturated solution of $NaHCO_3$ and the aqueous phase was extracted with DCM (3 times). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude mixture was purified by preparative HPLC to afford the compounds **25**, **26**, **28**, **36**, **46**, **47** as TFA salts.

General procedure 5 (GP5) for Acylation: Representative experimental procedure for the Acylation using heterocycles with the anhydride.

To solution of succinic anhydride (1 eq.) in anhydrous DCM was added the heterocycle base (1 eq) and the reaction mixture was stirred for 2 h at room temperature. The DCM was evaporated and the crude compound was recrystallized from Hex/EtOAc to afford the compounds **6**, **7**, **8**.

General procedure 6 (GP6) for Friedel Crafts reaction: Representative experimental procedure for the Friedel Crafts using heterocycles with the anhydride.

To solution of succinic anhydride (1 eq.) in anhydrous DCM was added anhydrous $AlCl_3$ (1.2 eq.) at 0 °C and the reaction mixture was stirred for 2 h at 0 °C. Then the aromatic heterocycle was added dropwise over a period of 1 h. The reaction mixture was stirred for 4 h at room temperature. 50 ml of ice-cold H_2O was added at 0 °C, followed by acidification with 2N HCl to pH 2. The aqueous phase was extracted with DCM (3 times). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude mixture was purified by recrystallization or flash column chromatography to afford the compounds **9**, **10**.

*Benzyl tert-butyl {6-[(2,2-diphenylethyl)amino]-6-oxohexane-1,5-diyl}(S)-dicarbamate (**1**)*

Compound **1** was prepared according to general procedure 1 (**GP1**). The crude mixture was purified by flash column chromatography (DCM/MeOH: 99/1) to afford compound **1** (2.37 g, 4.24 mmol, 80%) as a colorless solid. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 7.28-7.14 (m, 15H), 5.94 (br s, 1H), 5.01 (s, 2H), 4.92 (s, 1H), 4.71 (s, 1H), 4.10 (t, *J* = 8.0 Hz, 1H), 3.90-3.82 (m, 3H), 3.05-3.02 (m, 2H), 1.63-1.56 (m, 1H), 1.38-1.13 (m, 14H). ^{13}C-NMR (90 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.8 (C=O), 155.5 (C=O), 154.5 (C=O), 140.7 (2 x C_q), 135.6 (C_q), 127.73 and 127.73 (4 x CH, rotamers), 127.5 (2 x CH), 127.1 (2 x CH), 127.0 (4 x CH), 125.9 (CH), 125.5 (CH), 79.0 (C_q), 65.7 (CH_2), 53.3 (CH), 49.6 (CH), 42.7 (CH_2), 39.4 (CH_2), 31.0 (CH_2), 28.4 (CH_2), 27.3 (3 x CH_3), 21.3 (CH_2). MS (EI) m/z (%): 559 (3, M^+), 307 (24), 263(10), 218 (15), 180 (35), 174 (13), 167 (29), 108 (10), 91 (100), 84 (19), 79 (13), 59 (12), 57 (43.4), 56 (14), 44 (12), 41 (31), 39 (12), 30 (26), 28 (11) . MS (EI) m/z calcd for $C_{33}H_{41}N_3O_5$ $[M]^+$, 559.3; found, 559.3. HPLC purity, 98%, t_R = 22.3 min, gradient A. $[\alpha]^{25}_D$ -19.0 (c = 0.97, MeOH).

*tert-Butyl (S)-{6-amino-1-[(2,2-diphenylethyl)amino]-1-oxohexan-2-yl}carbamate (**2**)*

Compound **2** was prepared according to general procedure 3 (**GP3**). Compound **2** (1.82 g, 4.28 mmol, 99%) was afforded as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.23-7.17 (m, 4H), 7.16-7.13 (m, 6H), 6.29 (s, 1H), 4.96 (s, 1H), 4.11 (t, *J* = 8.0 Hz, 1H), 3.91-3.75 (m, 3H), 2.59-2.53 (m, 2H), 1.65-1.61 (m, 1H), 1.40-1.27 (m, 12H), 1.18-1.14 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.9 (C=O), 154.5 (C=O), 140.74 (C_q), 140.72 (C_q), 127.71 and 127.68 (4 x CH, rotamers), 127.0 (4 x CH), 125.8 (CH), 78.9 (C_q), 53.3 (CH), 49.6 (CH), 42.7 (CH_2), 40.5 (CH_2), 31.5 (CH_2), 28.7 (CH_2), 27.3 (3 x CH_3), 21.5 (CH_2). MS (EI) m/z (%): 425 (12, M^+), 258 (14, M^+-$C_{13}H_{11}$), 202 (18), 181 (11), 180 (25), 173 (14), 167 (27), 165 (18), 145 (13), 139 (15), 129 (12), 84 (100), 82 (18), 57 (50), 56 (18), 41 (26), 30 (36). MS (EI) m/z calcd for $C_{25}H_{36}N_3O_3$ $[M+H]^+$, 426.2;

found, 426.2. LC-MS (ESI) m/z calcd. for $C_{25}H_{36}N_3O_3$ $[M+H]^+$, 426.3; found, 426.3. HPLC purity, 95%, t_R = 11.0 min, gradient B. $[\alpha]^{24}_D$ -22.5 (c = 0.67, MeOH).

*(S)-2-[(benzyloxy)carbonyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**3**)*

To a 1 M aqueous solution of NaOH (3.20 g, 80.0 mmol in 80 mL of H_2O) at room temeparture were added (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5.00 g, 28.2) and dioxane slowly until the suspension dissolves. Then benzyl chloroformate was added (6.25 g, 5.59 µL, 36.7 mmol) dropwise over a period of 30 min at 0 °C. The reaction mixture was stirred for 16 h at room temperature. Dioxane was removed by evaporation and reaction mixture was acidified to pH 2 with 1M HCl at 0 °C. The aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford the title compound **3** (8.68, 27.9 mmol, 99%) as a colorless foam. ^{1}H-NMR (360 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 7.32-6.99 (m, 9H), 5.19-5.10 (m) and 4.91 (t, *J* = 4.9 Hz, 3H), 4.70 (d, *J* = 16.3 Hz), 4.50 (m, 1H), 3.22-3.07 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 175.4 and 175.0 (C=O, rotamers), 155.4 and 154.4 (C=O, rotamers), 135.2 (C_q), 131.9 (C_q), 131.2 (C_q), 130.4 and 130.3 (CH, rotamers), 127.5 (2 x CH), 127.2 and 127.1 (CH, rotamers), 127.0 and 126.9 (2 x CH, rotamers), 126.1 (CH), 126.0 (CH), 125.2 (CH), 66.8 and 66.6 (CH_2, rotamers), 52.4 and 52.0 (CH, rotamers), 43.5 and 43.3 (CH_2, rotamers), 30.2 and 29.8 (CH_2, rotamers). MS (EI) m/z (%): 222 (14, $[M+H]^+$ - $C_{11}H_{11}NO_4$), 220 (11, M^+-C_7H_7), 176 (53), 130 (23), 104 (11), 91 (100). MS (EI) m/z calcd for $C_{18}H_{17}NO_4$ $[M]^+$, 311.1; found, 311.1. LC-MS (ESI) m/z calcd. for $C_{18}H_{17}NO_4Na$ $[M+Na]^+$, 334.1; found, 334.6. HPLC purity, 96%, t_R = 20.5 min, Gradient A. $[\alpha]^{24}_D$ +23.6 (c = 1.03, MeOH).

*Benzyl (S)-3-{[(S)-5-((tert-butoxycarbonyl)amino)-6-((2,2-diphenylethyl)amino)-6-oxohexyl] carbamoyl}-3,4-dihydroisoquinoline-2(1H)-carboxylate (**4**)*

Compound **4** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 97/3) to afford compound **4** (1.2 g, 1.8 mmol, 51%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.34-7.11 (several m, 19H), 5.96 (br s, 1H), 5.15-5.10 (m, 2H), 4.60-4.65 (m, 2H), 4.50 (d, *J* = 15.8 Hz, 1H), 4.20 (t, *J* = 8.0 Hz, 1H), 3.77-3.74 (m, 2H), 3.69-3.64 (m, 1H), 3.08 (d, *J* = 5.5 Hz, 2H), 2.90-2.86 (m, 2H), 1.44-1.42 (m, 1H), 1.26-1.23 (m, 10H), 1.20-1.13 (2H), 1.05-0.99 (2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 171.0 (C=O), 169.9 (C=O), 154.6 (C=O), 140.7 (2 x C_q), 135.1 (C_q), 132.7 (C_q), 127.69 and 127.66 (4 x CH, rotames), 127.6 (2 x CH), 127.3 (2 x CH), 127.1 (2 x CH), 127.0 (4 x CH), 125.8 (2 x CH), 79.0 (C_q), 66.9 (CH_2), 55.4 (CH) 53.3 (CH), 49.6 (CH), 44.2 (CH_2), 42.7 (CH_2), 37.4 (CH_2), 30.3 (CH_2), 27.8 (CH_2), 27.3 (3 x CH_3), 21.1 (CH_2). LC-MS (ESI) m/z calcd. for $C_{43}H_{51}N_4O_6$ $[M+H]^+$, 719.4; found, 720.2. HPLC purity, 96%, t_R = 22.2 min, gradient A. $[\alpha]^{24}_D$ -18.7 (c = 0.51, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**5**)*

Compound **5** was prepared according to general procedure 3 (**GP3**). Compound **5** (2.0 g, 3.4 mmol, 98%) was afforded as a colorless solid. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 7.24-7.07 (m, 15H + $CDCl_3$, $H_{1\text{-}3,\ 6,\ 34\text{-}43}$), 6.97 (br s, 1H, H_{21}), 6.08 (br s, 1H, H_{26}), 4.97 (s, 1H), 4.12 (t, *J* = 8.0 Hz, 1H, H_{23}), 3.93-3.75 (m, 5H, $H_{8,\ 10,\ 22}$), 3.46 (dd, *J* = 5.1 Hz, *J* = 10.6 Hz, 1H, H_{18}), 3.17-3.12 (m, 3H, $H_{7,\ 14a}$), 2.74 (dd, *J* = 10.4 Hz, *J* = 16.3 Hz, 1H, H_{14b}), 1.59 (m, 1H, H_{17a}), 1.41-1.35 (m, 3H, $H_{15,\ 17b}$), 1.31 (s, 9H, $H_{31\text{-}33}$), 1.20-1.12 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 172.1 (C=O), 170.9 (C=O), 155.9 (C=O), 140.7 (2 x C_q), 134.8

(C_q), 133.3 (C_q), 128.1 (CH), 127.7 (4 x CH), 127.0 (4 x CH), 125.8 (2 x CH), 125.6 (CH), 125.1 (CH), 124.5 (CH), 78.9 (C_q), 55.5 (CH), 53.3 (CH), 49.6 (CH_2), 46.4 (CH), 42.7 (CH_2), 37.2 (CH_2), 30.8 (CH_2), 30.0 (CH_2), 28.0 (CH_2), 27.2 (3 x CH_3), 21.4 (CH_2). HRMS (ESI) m/z calcd for $C_{35}H_{45}N_4O_4$ $[M+H]^+$, 585.3435; found, 585.3436. MS (EI) m/z (%): 584 (3, M^+), 133 (14, $C_9H_{11}N$), 132 (100, $C_9H_{10}N$), 131 (20), 130 (11), 84 (11), 59 (32), 57 (12), 41 (17). MS (EI) m/z calcd for $C_{35}H_{44}N_4O_4$ $[M]^+$, 584.3; found, 584.3. HPLC purity, 97%, t_R = 19.5 min, gradient A. $[\alpha]^{24}_D$ -38.0 (c = 0.74, MeOH).

*4-oxo-4-(piperidin-1-yl)butanoic acid (**6**)*[27]

Compound 6 was prepared according to general procedure 5 (**GP5**). The crude solid mixture was purified by recrystallization (Hex/EtOAc: 1/2) to afford compound **6** (2.6 g, mmol, 94%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 3.50 (t, *J* = 6.0 Hz, 2H), 3.36 (t, *J* = 6.0 Hz, 2H), 2.62-2.61 (m, 4H), 1.60-1.48 (m, 6H). ^{13}C-NMR (90 MHz, $CDCl_3$): δ (ppm) 176.4 (C=O), 170.7 (C=O), 46.0 (CH_2), 43.4 (CH_2), 30.4 (CH_2), 28.5 (CH_2), 26.6 (CH_2), 25.8 (CH_2), 24.7 (CH_2).

*4-morpholino-4-oxobutanoic acid (**7**)*[28]

Compound **7** was prepared according to general procedure 5 (**GP5**). The crude solid mixture was purified by recrystallization (Hex/EtOAc: 1/3) to afford compound **7** (2.05 g, mmol, 95%) as light yellow crystals. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 3.64-56 (m, 6H), 3.44-3.42 (m, 2H), 2.67-2.62 (m, 2H), 2.59-2.55 (m, 2H). ^{13}C-NMR (90 MHz, $CDCl_3$): δ (ppm) 177.0 (C=O), 170.5 (=CO), 66.9 (CH_2), 66.6 (CH_2), 46.0 (CH_2), 42.4 (CH_2), 29.4 (CH_2), 27.9 (CH_2).

*4-(4-methylpiperazin-1-yl)-4-oxobutanoic acid (**8**)*[27x]

Compound **8** was prepared according to general procedure 5 (**GP5**). The crude product was purified by recrystallization (Hex/EtOAc/EtOH: 2/4/1) to afford the titled compound **8** (1.71 g, 8.54 mmol, 86%) as yellowish solid. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 10.69 (br s, 1H), 3.62 (t, *J* = 6.0 Hz, 2H), 3.52 (t, *J* = 6.0 Hz, 2H), 2.57-2.44 (m, 8H), 2.30 (s, 3H). ^{13}C-NMR (90 MHz, $CDCl_3$): δ (ppm) 175.2 (C=O), 169.5 (C=O), 66.9 (CH_2), 53.2 (CH_2), 52.3 (CH_2), 44.0 (CH_3), 43.4 (CH_2), 39.8 (CH_2), 28.9 (CH_2), 27.2 (CH_2).

*4-oxo-4-(thiophen-2-yl)butanoic acid (**9**)*[29]

Compound **9** was prepared according to general procedure 9 (**GP6**). The crude product was purified by recrystallization (H_2O) to afford compound **9** (1.38 mg, 7.5 mmol, 63%) as a light yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.70 (dd, *J* = 1.1, *J* = 3.8, 1H), 7.58 (dd, *J* = 1.1, *J* = 4.9, 1H), 7.07 (dd, *J* = 3.8, *J* = 4.9, 1H), 3.19 (t, *J* = 6.8 Hz, 2H), 2.74 (t, *J* = 6.7 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 190.9 (C=O), 178.5 (C=O), 143.7 (C_q), 134.0 (CH), 132.3 (CH), 128.4 (CH), 33.9 (CH), 28.2 (CH).

*4-(furan-2-yl)-4-oxobutanoic acid (**10**)*[30]

Compound **10** was prepared according to general procedure 6 (**GP6**). The crude product was purified by flash column chromatography (DCM/MeOH/AcOH: 96/4/0.1) to afford compound **10** (263 mg, 1.6 mmol, 57%) as a light yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.52 (dd, *J* = 0.8, *J* = 1.7, 1H), 7.16 (dd, *J* = 0.8, J = 3.6, 1H), 6.48 (dd, *J* = 1.7, *J* = 3.6, 1H), 3.11 (t, *J* = 6,7 Hz, 2H), 2.73 (t, *J* = 6.7 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 187.2 (C=O), 178.3 (C=O), 152.5 (C_q), 146.6 (CH), 117.3 (CH), 112.5 (CH), 33.0 (CH), 27.7 (CH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**11**)*

Compound **11** was prepared according to general procedure 2 (**GP2**) by using benzoyl propionic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 8/3/1) to afford Compound **11** (89 mg, 0.12 mmol, 70%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.94-7.92 (m, 2H, H_{44} and H_{48}), 7.63-7.58 (m, 1H, H_{46}), 7.50-7.47 (m, 2H, H_{45} and H_{47}), 7.24-7.13 (m, 15H, H_1- H_3, H_6, H_{27}, H_{33}-H_{42}), 5.96-5.95 (br s, 1H, H_{25}, H_{19}), 4.91 (br s, 1H, H_8), 4.80 (d, J = 15.9 Hz, 1H, H_{10a}), 4.57 (br s, 1H, H_{10b}), 4.19 (t, J = 7.7 Hz, 1H, H_{30}), 3.76-3.68 (m, 2H, H_{29a} and H_{24}), 3.66-3.64 (m, 1H, H_{29b}), 3.27 (t, J = 6.4 Hz, 2H, H_{16}), 3.18 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.06 (br s, 1H, H_{7b}), 2.96-2.75 (m, 4H + H_2O, H_{15} and H_{20}), 1.44-1.39 (m, 1H, H_{23a}), 1.38 (s, 9H, H_{53}-H_{55}), 1.36-1.29 (m, 1H, H_{23b}), 1.23-1.16 (m, 2H, H_{21}), 1.10-1.00 (m, 2H, H_{22}). ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.93-7.90 (m, 2H), 7.52-7.51 (m, 1H,), 7.42-7.39 (m, 2H), 7.21-7.10 (m, 15H), 6.64 (t, J = 5.9 Hz, rotamer 1) and 6.13 (t, J = 5.9 Hz, 1H, rotamer 2), 6.45 (br s, rotamer 1) and 6.25 (br s, 1H, rotamer 2), 5.15 (br s, 1H), 5.00-4.53 (several m, 3H), 4.09-4.04 (m, 1H), 3.85-3.60 (m, 4H), 3.49-3.41 (m, 1H), 3.29-2.71 (several m, 5H), 2.56-2.50 (m, 1H), 1.49-1.41 (m, 1H), 1.32 (s, 9H), 1.28-0.93 (several m, 5H). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.5 (C=O), 143.3 (2 x C_q, C_{31-32}), 137.6 (C_q), 133.4 (C_q, C_{46}), 128.8 (4 x CH), 128.42 and 128.40 (4 x CH, rotamers), 128.3 (CH), 126.8 (2 x CH), 126.5 (CH), 78.6 (C_q), 50.7 (CH, C_{30}), 43.8 (CH_2, C_{29}), 34.0 (CH_2, C_{16}), 32.3 (CH_2, C_{21}), 29.2 (CH_2, C_{23}), 28.7 (3 x CH_3, C_{53-55}), 28.2 (CH_2, C_{15}), 23.0 (CH_2, C_{22}). Four C=O, One C_q, two CH, four aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. All protons and carbons have been assigned by performing 2D NMR analysis [COSY (400 K), HSQC (350 K) and HMBC (350 K)], where the assigned structure and 2D spectra of compound **11** can be

found in supporting information. HRMS (ESI) m/z calcd for $C_{45}H_{52}N_4O_6$ Na $[M+Na]^+$, 767.3779; found, 767.3789. HPLC purity, 100%, t_R = 22.7min, gradient A. $[\alpha]^{25}_D$ -23.5 (c = 0.69, MeOH).

*tert-Butyl{(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxopentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**12**)*

Compound **12** was prepared according to general procedure 2 (**GP2**) by using 4-oxopentanoic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 6/3/1) to afford compound **12** (45 mg, 0.066 mmol, 78%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 350 K): δ (ppm) 7.33-7.19 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 6.02 (br s, 1H, $H_{12,\ 26}$), 4.93 (br s, 1H, H_8), 4.80 (d, J = 16.0 Hz, 1H, H_{10a}), 4.60 (br s, 1H, H_{10b}), 4.26 (t, J = 8.0 Hz, 1H, H_{23}), 3.84-3.79 (m, 2H, $H_{18,\ 22a}$), 3.75-3.71 (m, 1H, H_{22b}), 3.27-3.16 (m, 2H, H_{47}), 3.08-2.96 (m, 2H, H_7), 2.76-2.57 (m, 4H, $H_{14,\ 45}$), 2.16 (s, 3H, H_{49}), 1.51-1.41 (m, 10H, $H_{31\text{-}33,\ 17a}$), 1.37-1.30 (m, 1H, H_{17b}), 1.27-1.21 (m, 2H, H_{15}), 1.11-1.04 (m, 2H, H_{16}).^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.4 (C=O), 170.5 (C=O), 167.9 (C=O), 157.3 (C=O), 143.2 (2 x C_q), 128.76 and 128.73 (4 x CH, rotamers), 128.70 (CH), 128.33 and 128.31 (4 x CH, rotamers), 126.7 (2 x CH), 126.4 (CH), 78.5 (C_q), 50.6 (CH), 50.0 (CH), 47.9 (CH_2), 43.7 (CH_2), 38.3 (CH_2), 32.2 (CH_2), 30.4 (CH_2), 29.1 (CH_3), 28.7 (3 x CH_3), 22.8 (CH_2). One C=O, two C_q, one CH, two aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{40}H_{50}N_4O_6$Na $[M+Na]^+$, 705.3623; found, 705.3625. LC-MS (ESI) m/z calcd. for $C_{40}H_{51}N_4O_6$ $[M+H]^+$, 683.4; found, 683.7. HPLC purity, 95%, t_R = 12.9 min, gradient B.

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-6-[(S)-2-(4-(4-fluorophenyl)-4-oxobutanoyl)-1,2,3,4 -tetrahydroisoquinoline-3-carboxamido]-1-oxohexan-2-yl}carbamate (**13**)*

Compound **13** was prepared according to general procedure 2 (**GP2**) by using 3-(4-fluorobenzoyl)propionic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 6/3/1) to afford compound **13** (39 mg, 0.05 mmol, 60%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 8.09 -8.07 (m, 2H, $H_{51, 55}$), 7.29-7.20 (m, 16H, $H_{1\text{-}3, 6, 21, 34\text{-}43, 52, 54}$), 6.03 (br s, 1H, $H_{12, 26}$), 4.97 (br s, 1H, H_8), 4.86 (d, J = 15.9 Hz, 1H, H_{10a}), 4.64 (br s, 1H, H_{10b}), 4.26 (t, J = 7.7 Hz, 1H, H_{23}), 3.84-3.80 (m, 2H, $H_{18, 22a}$), 3.72 (m, 1H, H_{22b}), 3.33 (t, J = 7.2 Hz, 2H, H_{47}), 3.25 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.12 (br s, 1H, H_{7b}), 2.95-2.88 (m, 4H + H_2O, $H_{14, 45}$), 1.44-1.31 (m, 11H, $H_{17, 31\text{-}33}$), 1.29-1.20 (m, 2H, H_{15}), 1.11-1.05 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K): δ (ppm) 158.9 (C=O) 143.3 (2 x C_q), 140.0 (C_q), 131.3 (2 x C_q), 128.8 (4 x CH), 128.4 (4 x CH), 126.8 (2 x CH), 126.5 (CH), 116.0 (2 x CH), 55.1 (CH), 50.7 (CH), 43.8 (CH_2), 33.9 (CH_2), 32.3 (CH_2), 31.6 (CH_2), 29.2 (CH_2), 28.7 (3 x CH_3), 23.0 (CH_2). Four C=O, One CF, One CH, for aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{45}H_{53}FN_4O_6$ $[M+H]^+$, 763.3865; found, 763.3865. HPLC purity, 99%, t_R = 21.8 min, gradient A. $[\alpha]^{24}_D$ – 34.0 (c = 0.5, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-6-[(S)-2-(4-(4-methoxyphenyl)-4-oxobutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]-1-oxohexan-2-yl}carbamate (**14**)*

14 was prepared according to general procedure 2 (**GP2**) by using 3-(4-methoxybenzoyl)propionic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 6/3/1) to afford **14** (39 mg, 0.05 mmol, 58%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.95-7.94 (m, 2H, $H_{51, 55}$),

7.28-7.16 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 7.04-7.02 (m, 2H, $H_{52,\ 54}$), 5.98 (br s, 1H, $H_{12,\ 26}$), 4.94 (br s, 1H, H_8), 4.83 (d, J = 16.0 Hz, 1H, H_{10a}), 4.60 (br s, 1H, H_{10b}), 4.22 (t, J = 8.0 Hz, 1H, H_{23}), 3.87 (s, 3H, H_{57}), 3.80-3.77 (m, 2H, $H_{18,\ 22a}$), 3.71-3.68 (m, 1H, H_{22b}), 3.25 (t, J = 7.2 Hz, 2H, H_{47}), 3.21 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.08 (br s, 1H, H_{7b}), 2.94-2.75 (m, 4H + H_2O, $H_{14,\ 45}$), 1.44-1.27 (m, 11H, $H_{17,\ 31\text{-}33}$), 1.26-1.19 (m, 2H, H_{15}), 1.08-1.01(m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 171.6 (C=O), 169.6 (C=O), 162.8 (C-OMe), 154.6 (C=O), 142.3 (2 x C_q), 133.0 (C_q), 129.7 (2 x CH), 129.5 (C_q), 127.9 and 127.87 (4 x CH, rotamers), 127.47 and 127.45 (4 x CH, rotamers), 125.8 (2 x CH), 125.6 (CH), 113.6 (2 x CH), 77.7 (C_q), 55.1 (CH_3), 49.8 (CH), 42.8 (CH_2), 32.7 (CH_2), 31.4 (CH_2), 28.2 (CH_2), 27.8 (3 x CH_3), 27.7 (CH_2), 22.0 (CH_2). Two C=O, one C_q, four aromatic CH, two CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{46}H_{54}N_4O_7Na$ $[M+Na]^+$, 797.3885; found, 797.3888. HPLC purity, 99%, t_R = 21.8 min, gradient A. $[\alpha]^{22}_D$ - 23. 5 (c = 0.46, MeOH).

tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-6-[(S)-2-(4-(4-(methylsulfonyl)phenyl)-4-oxobutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]-1-oxohexan-2-yl}carbamate

(15)

Compound **15** was prepared according to general procedure 2 (**GP2**) by using 3-(4-methylsulfonylbenzoyl)propionic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 3/3/1) to afford compound **15** (56 mg, 0.068 mmol, 80%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 8.21-8.19 (m, 2H, $H_{51,\ 55}$), 8.11-8.09 (m, 2H, $H_{52\text{-}54}$), 7.29-7.06 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 6.03 (br s, 1H, $H_{12,\ 26}$), 4.96 (br s, 1H, H_8), 4.86 (d, J = 16.0 Hz, 1H, H_{10a}), 4.64 (br s, 1H, H_{10b}), 4.26 (t, J = 8.0 Hz, 1H, H_{23}), 3.86-3.81 (m, 2H, $H_{18,\ 22a}$), 3.74-3.71 (m, 1H, H_{22b}), 3.38 (t, J = 7.1 Hz, 2H, H_{47}),

3.23 (m, 4H, $H_{7a, 57}$), 3.10 (br s, 1H, H_{7b}), 2.98-2.86 (m, 4H + H_2O, $H_{14, 45}$), 1.40-1.30 (m, 11H, $H_{17, 31-33}$), 1.28-1.22 (m, 2H, H_{15}), 1.11-1.06 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 199.3 (C=O), 172.5 (C=O), 171.9, 171.6, 155.6 (C=O), 145.0 (CS), 143.3 (2 x C_q), 141.3 (C_q), 129.3 (2 x CH), 128.87 and 128.84 (4 x CH, rotamers), 128.43 and 128.41 (4 x CH, rotamers), 127.8 (2 x CH), 126.8 (2 x CH), 126.5 (CH) , 126.3 (CH), 126.1 (CH), 78.7 (C_q), 56.7 (CH), 55.1 (CH), 50.7 (CH), 47.3 (CH_2), 44.0 (CH_3) 43.8 (CH_2), 38.8 (CH_2), 34.4 (CH_2) , 32.4 (CH_2), 31.7 (CH_2), 29.3 (CH_2), 28.8 (3 x CH_3), 28.4 (CH_2), 23.1 (CH_2). Two C_q and two aromatic CH groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{46}H_{54}N_4O_8SNa$ $[M+Na]^+$, 845.3555; found, 845.3557. LC-MS (ESI) m/z calcd. for $C_{46}H_{55}N_4O_8S$ $[M+H]^+$, 824.0; found, 824.4. HPLC purity, 96%, t_R = 21.0 min, gradient A. $[\alpha]^{24}_D$ -19.8 (c = 0.20, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-(piperidin-1-yl)butanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**16**)*

Compound **16** was prepared according to general procedure 2 (**GP2**) by using compound **6.** The crude product was purified by flash column chromatography (DCM/MeOH: 97/3) to afford compound **16** (58 mg, 0.077 mmol, 91%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.26-7.12 (m, 15H, $H_{1-3, 6, 21, 34-43}$), 5.94 (br s, 1H, $H_{12, 26}$), 4.95 (br s, 1H, H_8), 4.79 (d, J = 15.9 Hz, 1H, H_{10a}), 4.55 (br s, 1H, H_{10b}), 4.19 (t, J = 7.7 Hz, 1H, H_{23}), 3.80-3.72 (m, 2H, $H_{18, 22a}$), 3.68-3.64 (m, 1H, H_{22b}), 3.41 (m, 4H, $H_{51, 55}$), 3.22 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 2.99 (br s, 1H, H_{7b}), 2.92-2.83 (m, 2H, H_{47}), 2.78-2.58 (m, 4H, $H_{14, 45}$), 1.61-1.57 (m, 2H, H_{53}), 1.49-1.44 (m, 4H, $H_{52, 54}$), 1.41-1.34 (m, 10H, $H_{17a, 31-33}$), 1.31-1.23 (m, 1H, H_{17b}), 1.20-1.15 (m, 2H, H_{15}), 1.03-0.97 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 171.6 (C=O), 169.4 (C=O), 154.5

(C=O), 142.3 (2 x C_q), 127.92 and 127.89 (4 x CH, rotamers), 127.49 and 127.47 (4 x CH, rotamers), 126.2 (CH), 125.9 (CH), 125.8 (2 x CH), 125.6 (CH), 77.7 (C_q), 54.1 (CH), 51.8 (CH), 49.8 (2 x CH_2), 45.0 (CH_2), 42.9 (CH_2), 38.2 (CH_2), 31.4 (CH_2), 30.4 (CH_2), 28.3 (CH_2), 27.8 (3 x CH_3), 23.6 (2 x CH_2), 22.0 (CH_2), 21.5 (CH_2). Two C=O, two C_q, one CH, one aromatic CH, and two CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{44}H_{58}N_5O_6$ $[M+H]^+$, 752.4382; found, 752.4379. LC-MS (ESI) m/z calcd. for $C_{44}H_{57}N_5O_6Na$ $[M+Na]^+$, 775.0; found, 774.6. HPLC purity, 98%, t_R = 13.2 min, gradient B. $[\alpha]^{24}_D$ -15.3 (c = 0.31, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-6-[(S)-2-(4-morpholino-4-oxobutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]-1-oxohexan-2-yl}carbamate (**17**)*

Compound **17** was prepared according to general procedure 2 (**GP2**) by using compound **7** The crude product was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **17** (54 mg, 0.072 mmol, 83%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.25-7.13 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 5.96-5.94 (m, 1H, $H_{12,\ 26}$), 4.93 (br s, 1H, H_8), 4.79 (d, J = 15.8 Hz, 1H, H_{10a}), 4.54 (br s, 1H, H_{10b}), 4.19 (t, J = 7.7 Hz, 1H, H_{23}), 3.79-3.72 (m, 2H, $H_{18,\ 22a}$), 3.69-3.64 (m, 1H, H_{22b}), 3.56 (m, 4H, $H_{51,\ 55}$), 3.45 (m, 4H, $H_{52,\ 54}$), 3.21 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.00 (br s, 1H, H_{7b}), 2.89-2.86 (m, 2H, H_{47}), 2.75-2.54 (m, 4H, $H_{14,\ 45}$), 1.40-1.34 (m, 10H, $H_{17a,\ 31\text{-}33}$), 1.29-1.23 (m, 1H, H_{17b}), 1.20-1.15 (m, 2H, H_{15}), 1.03-0.98 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.5 (C=O), 171.2 (C=O), 170.4 (C=O), 155.5 (C=O), 143.3 (2 x C_q), 134.0 (C_q), 133.8 (C_q), 128.86 and 128.82 (4 x CH, rotamers), 128.42 and 128.40 (4 x CH, rotamers), 127.2 (CH), 126.79 and 126.76 (2 x CH), 126.5 (CH), 78.6 (C_q), 66.6 (2 x CH_2), 55.30 and 55.27 (CH, rotamers), 52.3 (CH), 50.7 (CH), 46.0 (CH_2), 43.8 (CH_2), 39.0

(CH_2), 32.3 (CH_2), 31.3 (CH_2), 29.2 (CH_2), 28.7 (3 x CH_3), 28.6 (CH_2), 22.9 (CH_2). One C=O, two aromatic CH, four CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{43}H_{55}N_5O_7Na$ $[M+Na]^+$, 776.3994; found, 776.4016. LC-MS (ESI) m/z calcd. for $C_{44}H_{56}N_5O_7$ $[M+H]^+$, 754.9; found, 754.5. HPLC purity, 99%, t_R = 12.9 min, gradient B.

*4-{4-[(S)-3-(((S)-5-((tert-butoxycarbonyl)amino)-6-((2,2-diphenylethyl)amino)-6-oxohexyl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-4-oxobutanoyl]-1-methylpiperazin-1-ium 2,2,2-trifluoroacetate (**18**)*

Compound **18** was prepared according to general procedure 2 (**GP2**) by using compound **8**. The crude product was purified by preparative HPLC (t_r = 12.4 min, Method B) to afford compound **18** (48 mg, 0.063 mmol, 73%) as colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.26-7.13 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 5.98 (br s, 1H, $H_{12,\ 26}$), 4.91 (br s, 1H, H_8), 4.78 (d, J = 15.8 Hz, 1H, H_{10a}), 4.55 (br s, 1H, H_{10b}), 4.20 (t, J = 7,7 Hz, 1H, H_{23}), 3.77-3.66 (m, 7H, $H_{18,\ 22a,\ 51,\ 55}$), 3.20 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.10-3.03 (m, 5H, $H_{7b,\ 52,\ 54}$) 2.89-2.85 (m, 2H, H_{47}), 2.75-2.63 (m, 7H, $H_{14,\ 45,\ 56}$), 1.40-1.32 (m, 10H, $H_{17a,\ 31\text{-}33}$), 1.28-1.24 (m, 1H, H_{17b}), 1.20-1.15 (m, 2H, H_{15}), 1.06-0.95 (m, 2H, H_{15}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.5 (C=O), 171.4 (C=O), 170.5 (C=O), 155.6 (C=O), 143.3 (2 x C_q), 133.8 (C_q), 133.7 (C_q), 128.86 and 128.83 (4 x CH, rotamers), 128.43-128.41 (4 x CH, rotamers), 127.2 (CH), 126.8 (2 x CH), 126.5 (CH), 78.7 (C_q), 55.1 (CH), 53.1 (2 x CH_2), 50.7 (CH), 46.0 (CH), 43.8 (CH_2), 43.0 (CH_3), 39.0 (CH_2), 32.3 (CH_2), 31.5 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 28.7 (3 x CH_3), 28.2 (CH_2) 22.9 (CH_2). One C=O, one CH, two aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{44}H_{59}N_6O_6$ $[M+H]^+$,

767.4491; found, 767.4489. LC-MS (ESI) m/z calcd. for $C_{44}H_{59}N_6O_6$ $[M+H]^+$, 767.5; found, 767.6. HPLC purity, 95%, t_R = 11.6 min, gradient B. $[\alpha]^{24}_D$ -16.8 (c = 0.51, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-(thiophen-2-yl)butanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**19**)*

Compound **19** was prepared according to general procedure 2 (**GP2**) by using compound **9**. The crude product was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **19** (45 mg, 0.060 mmol, 70%) as colorless solid ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K, rotamers were observed): δ (ppm) 8.08 (s) and 7.85 (d, *J* = 3.8 Hz, 1H, rotamers, H_{53}), 7.83 (d, *J* = 5.0 Hz, 1H, H_{51}), 7.25-7.13 (m, 16H, $H_{1\text{-}3,\ 6,\ 23,\ 29\text{-}38,\ 52}$), 5.89 (br s, 1H, $H_{15,\ 21}$), 4.91 (br s, 1H, H_8), 4.79 (d, J = 15.8 Hz, 1H, H_{10a}), 4.57 (br s, 1H, H_{10b}), 4.20 (t, *J* = 7.7 Hz, 1H, H_{26}), 3.78-3.74 (m, 2H, $H_{20,\ 25a}$), 3.70-3.68 (m, 1H, H_{25b}), 3.24 (t, *J* = 6.55, 2H, H_{40}), 3.21 (dd, *J* = 4.0 Hz, *J* =15.7 Hz, 1H, H_{7a}), 3.04 (br s, 1H, H_{7b}), 2.92-2.71 (several m, 4H + H_2O, $H_{16,\ 39}$), 1.45-1.17 (m, 13H, $H_{17,\ 18,\ 47\text{-}49}$), 1.09-0.95 (m, 2H, H_{18}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 191.4 (C=O), 171.5 (C=O), 171.0 (C=O), 169.5 (C=O), 154.5 (C=O), 143.3 (2 x C_q), 142.2 (C_q), 133.5 (C_q), 132.1 (CH), 127.9 (CH), 127.82 and 128.79 (4 x CH, rotamers), 127.47 (CH), 127.41 and 127.35 (4 x CH, rotamers), 126.3 (CH), 125.8 (2 x CH), 125.5 (CH), 77.6 (C_q), 54.1 (CH), 53.8 (CH), 49.7 (CH), 45.0 (CH_2), 42.9 (CH_2), 38.1 (CH_2), 33.6 (CH_2), 31.4 (CH_2), 30.4 (CH_2), 27.8 (CH_2), 27.7 (3 x CH_3), 27.2 (CH_2), 21.9 (CH_2). One C_q, two aromatic CH groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{43}H_{50}N_4O_6Na$ $[M+Na]^+$, 773.3343; found, 773.3341. LC-MS (ESI) m/z calcd. for $C_{43}H_{51}N_4O_6S$ $[M+H]^+$, 752.0; found, 751.8. HPLC purity, 99%, t_R = 13.0 min, gradient B. $[\alpha]^{24}_D$ -23.6 (c = 0.77, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-6-[(S)-2-(4-(furan-2-yl)-4-oxobutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]-1-oxohexan-2-yl}carbamate (**20**)*

Compound **20** was prepared according to general procedure 2 (**GP2**) by using compound **10**. The crude product was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **20** (29 mg, 0.004 mmol, 43%) as colorless solid ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K, rotamers were observed): δ (ppm) 8.08 (s) and 7.79 (d, J = 1.7, 1H, rotamers H_{53}), 7.28 (d, J = 3.6, 1H, H_{51}), 7.25-7.13 (m, 15H, $H_{1\text{-}3,\ 6,\ 23,\ 29\text{-}38}$), 6.61 (s, 1H, H_{52}), 5.89 (br s, 1H, $H_{16,\ 21}$), 4.90 (br s, 1H, H_8), 4.78 (d, J = 15.9, 1H, H_{10a}), 4.55 (br s, 1H, H_{10b}), 4.20 (t, J = 7.7, 1H, H_{26}), 3.81-3.74 (m, 2H, $H_{20,\ 25a}$), 3.70-3.65 (m, 1H, H_{25b}), 3.19 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.12 (t, J = 6.6, 2H, H_{40}), 3.05 (br s, 1H, H_{7b}), 2.93-2.78 (m, 4H + H_2O, $H_{16,\ 39}$), 1.45-1.37 (m, 1H, H_{19a}), 1.34 (s, 9H, $H_{47\text{-}49}$), 1.32-1.24 (m, 1H, H_{19b}), 1.22-1.14 (m, 2H, H_{17}), 1.05-0.96 (m, 2H, H_{18}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.0 (C=O), 152.2 (C_q), 147.0 (CH), 142.7 (2 x C_q), 128.27 and 128.24 (4 x CH, rotamers), 127.88 and 127.86 (4 x CH, rotamers), 126.2 (CH), 125.9 (2 x CH), 117.4 (CH), 112.2 (CH), 78.1 (C_q), 50.2 (CH), 43.3 (CH_2), 33.2 (CH_2), 31.8 (CH_2), 28.7 (CH_2), 28.2 (3 x CH_3), 27.3 (CH_2), 22.4 (CH_2). Four C=O, two C_q, two CH, three aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{43}H_{51}N_4O_7$ $[M+H]^+$, 735.3752; found, 735.3757. LC-MS (ESI) m/z calcd. for $C_{43}H_{51}N_4O_7$ $[M+H]^+$: 735.38; found, 735.36. HPLC purity, 96%, t_R = 21.2 min. A different column was used in LC-MS measurement. Column: Kinetex 2.6u C8 100A, 75 × 2.1 mm, 2.6 µm particles, flow rate 0.3 mL /min, detection wavelengths: 254 nm. Solvent: Methanol (MeOH), water 0.1% HCOOH. Gradient: 0-10% MeOH in 0-3 min, 10-100% MeOH in 3-8%, 100% MeOH in 9%, 100-0% MeOH in 9-12 min.

*tert-Butyl {(S)-6-[(S)-2-(4-(3,4-dimethoxyphenyl)-4-oxobutanoyl)-1,2,3,4-tetrahydroisoquinoline -3-carboxamido]-1-[(2,2-diphenylethyl)amino]-1-oxohexan-2-yl}carbamate (**21**)*

Compound **21** was prepared according to general procedure 2 (**GP2**) by using 3-(4-dimethoxybenzoyl)propionic acid. The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **21** (52 mg, 0.065 mmol, 76%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K, rotamers were observed): δ (ppm) 7.68 and 7.67 (2 x d, J = 2.1 Hz each, 1H, rotamers, H_{48}), 7.55 (d, J = 2.1 Hz, 1H, H_{44}), 7.32-7.19 (m, 15H, $H_{1\text{-}3,\ 6,\ 19,\ 33\text{-}42}$), 7.11 and 7.10 (2 x s, 1H, rotamers, H_{47}), 6.02 (br s, 1H, $H_{19,\ 25}$), 4.98 (s, 1H, H_8), 4.87 (d, J = 15.9, 1H, H_{10a}), 4.64 (br s, 1H, H_{10b}), 4.26 (t, J = 8.0 Hz, 1H), 3.91 (s, 3H, H_{59}), 3.88 (s, 3H, H_{57}), 3.84-3.79 (m, 2H, $H_{24,\ 29a}$), 3.75-3.70 (m, 1H, H_{29b}), 3.31 (t, J = 6.5 Hz, 2H, H_{16}), 3.25 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.10 (br s, 1H, H_{7b}), 2.98-2.77 (m, 4H + H_2O, $H_{15,\ 20}$), 1.46-1.41(m, 1H, H_{23a}), 1.40 (s, 9H, 53-55), 1.37-1.23 (m, 3H, $H_{21,\ 23b}$), 1.12-1.09 (m, 2H, H_{22}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm): 197.5 (C=O), 172.0 (C=O), 171.6 (C=O), 170.0 (C=O), 155.1 (C=O), 152.0 (C-OMe), 148.5 (C-OMe), 142.7 (2 xC_q), 133.4 (C_q), 133.3 (C_q), 129.6 (C_q), 128.32 and 128.26 (4 x CH, rotamers) 127.82 and 127.80 (4 x CH, rotamers), 127.6 (CH), 126.7 (2 x CH), 126.5 (CH), 126.2, 126.0, 122.5 (CH), 110.9 (CH), 111.2 (CH), 77.8 (C_q), 55.7 (CH_3), 55.5 (CH_3), 54.1 (CH), 52.4 (CH), 50.0 (CH) 45.3 (CH_2), 43.0 (CH_2), 38.1 (CH_2), 32.8 (CH_2), 31.6 (CH_2), 30.9 (CH_2), 28.7 (CH_2), 28.1 (3 x CH_3), 27.6 (CH_2), 22.0 (CH_2). Few peaks in the aromatic area could not be assigned, due to overlapping peaks. HRMS (ESI) m/z calcd for $C_{47}H_{56}N_4O_8Na$ $[M+Na]^+$, 827.3990; found, 827.3997. LC-MS (ESI) m/z calcd. for $C_{47}H_{57}N_4O_8$ $[M+H]^+$, 806.0; found, 806.1. HPLC purity, 100%, t_R = 21.3 min, gradient A.

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**22**)*

Compound **22** was prepared according to general procedure 2 (**GP2**) by using 4-(phenyl)butyric acid. The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **22** (59 mg, 0.081 mmol, 95%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.30-7.18 (m, 20H, $H_{1\text{-}3,\ 6,\ 27,\ 33\text{-}42,\ 43\text{-}47}$), 6.02 (br s, 1H, $H_{19,\ 25}$), 4.90 (br s, 1H, H_8), 4.76 (br s, 1H, H_{10a}), 4.55 (d, J = 16.6 Hz, 1H, H_{10b}), 4.26 (t, J = 7.7 Hz, 1H, H_{30}), 3.87-3.79 (m, 2H, $H_{24,\ 29a}$), 3.75-3.71 (m, 1H, H_{29b}), 3.20 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.09 (br s, 1H, H_{7b}), 2.95-2.90 (m, 2H + H_2O, H_{20}), 2.70 (t, J = 7.6 Hz, 2H, H_{15}), 2.54-2.42 (m, 2H + DMSO, H_{17}), 1.97-192 (m, 2H, H_{16}), 1.44-1.30 (m, 11H, $H_{23,52\text{-}54}$), 1.25-1.21 (m, 2H, H_{21}), 1.11-1.05 (m, 2H, H_{22}). ^{13}C-NMR (150 MHz, DMSO-d_6, 300 K, rotamers were observed): δ (ppm) 173.0 (C=O), 171.1 (C=O), 170.8 (C=O), 156.0 (C=O), 143.6 (2 x C_q), 142.9 (C_q), 134.6 (C_q), 129.3, 129.23, 129.20, 129.16, 128.79, 128.77, 128.70, 128.6, 127.3, 127.2, 126.8, 126.7, 125.8, 78.8 (C_q), 56.0 (CH), 55.1 (CH), 53.4 (CH), 51.0 (CH_2), 46.2 (CH_2), 43.9 (CH_2), 35.6 (CH_2), 33.4 (CH_2), 32.5 (CH_2), 29.7 (CH_2), 29.1 (3 x CH_3), 27.3 (CH_2), 23.3 (CH_2). One CH_2 group was not detected due to peak broadening or overlapping caused by the high temperature. Few peaks in the aromatic area could not be assigned, due to overlapping peaks. HRMS (ESI) m/z calcd for $C_{45}H_{54}N_4O_5Na$ $[M+Na]^+$, 753.3986; found, 753.3994. HPLC purity, 98%, t_R = 21.5 min, gradient A.

*tert-Butyl{(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(3-phenylpropanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**23**)*

To a solution of 3-phenylpropionic acid (29 mg, 0.19 mmol) in 2 mL of anhydrous DMF were added diisopropylethylamine (34 mg, 45 µl, 0.26 mmol) and HATU (52 mg, 0.22

mmol) at 0 °C. The reaction mixture stirred for 30 min. Then the reaction mixture warmed up at rt and compound **5** (100 mg, 0.17 mmol) was added and stirred for 16h. The reaction mixture was washed with H_2O, then a saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with DCM. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 6/3/1) to afford the titled compound **23** (77 mg, 0.11 mmol, 63%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K, rotamers were observed): δ (ppm) 7.64-7.16 (m, 20H, $H_{1\text{-}3,\ 6,\ 23,\ 29\text{-}38,\ 49\text{-}53}$), 6.29 (br s, 1H, $H_{15,\ 21}$), 5.03-4.46 (m, 3H, $H_{8,\ 10}$), 4.24 (t, J = 7.8 Hz, 1H, H_{26}), 3.83 and 3.68 (2 x ddd, each J = 5.8 Hz, J = 7.7 Hz, J = 13.2 Hz, 2H, rotamers, H_{25}), 3.78 (br s, 1H, H_{20}), 3.31-2.75 (m, 8H + H_2O, $H_{7,\ 16,\ 39,\ 40}$), 1.42-1.24 (m, 11H, $H_{19,\ 46\text{-}48}$), 1.16-1.12 (m, 2H, H_{17}), 1.03-0.97 (m, 2H, H_{18}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.5 (C=O), 155.5 (C=O), 143.3 (2 x C_q), 142.0 (C_q), 128.87 and 128.83 (4 x CH, rotamers), 128.7 (2 x CH), 128.43 and 128.41 (4 x CH, rotamers), 126.8 (2 x CH), 126.3 (CH) 78.6 (C_q), 50.7 (CH), 43.8 (CH_2), 39.0 (CH_2), 35.2 (CH_2), 32.3 (CH_2), 31.0 (CH_2), 29.3 (CH_2), 28.8 (3 x CH_3), 22.9 (CH_2). Three C=O, two C_q, three CH, six aromatic CH and two CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{44}H_{52}N_4O_5Na$ $[M+Na]^+$, 739.3830; found, 739.3842. LC-MS (ESI) *m/z* calcd. for $C_{45}H_{52}N_4O_5Na$ $[M+Na]^+$, 739.4; found, 740.1. HPLC purity, 98%, t_R = 22 min, gradient A. $[\alpha]^{24}_D$ -23.3 (c = 0.70, MeOH).

*tert-Butyl {(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(5-oxo-5-phenylpentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**24**)*

Compound **24** was prepared according to general procedure 2 (**GP2**) by using 4-benzoylboutyric acid. The crude solid mixture was purified by preparative HPLC employing

method C (t_R = 19 min) to afford compound **24** (107 mg, 0.14 mmol, 45%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.98-7.96 (m, 2H, $H_{45, 49}$), 7.65-7.62 (m, 1H, H_{47}), 7.55-7.53 (m, 2H, $H_{46, 48}$), 7.32-7.19 (m, 15H, H $H_{1\text{-}3, 6, 23, 29\text{-}38}$), 6.03 (br s, 1H, $H_{15, 21}$), 4.93 (br s, 1H, H_8), 4.80 (d, J = 15.9 Hz, 1H, H_{10a}), 4.58 (br s, 1H, H_{10b}), 4.26 (t, J = 7.7 Hz, 1H, H_{26}), 3.87-3.79 (m, 2H, $H_{20, 25a}$), 3.75-3.71 (m, 1H, H_{25b}), 3.18 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.11-3.09 (m, 3H, $H_{7b, 41}$), 2.97-2.92 (m, 2H, H_{16}), 2.61-2.59 (m, 2H + DMSO, H_{39}), 2.0 (quint, J = 7.1 Hz, 2H, H_{40}), 1.45-1.31 (m, 11H, $H_{19, 54\text{-}56}$), 1.27-1.21 (m, 2H, H_{17}), 1.11-1.05 (m, 2H, H_{18}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 200.5 (C=O), 172.6 (C=O), 155.6 (C=O), 143.3 (2 x C_q), 137.5 (C_q), 133.4 (C_q), 129.2 (2 x CH), 128.86 and 128.8 (4 x CH, rotamers), 128.43 and 128.41 (4 x CH, rotamers), 128.3 (2 x CH), 126.8 (2 x CH), 78.6 (C_q) 55.1 (CH), 50.7 (CH), 46.0 (CH_2), 43.8 (CH_2) , 39.0 (CH_2) 38.1 (CH_2), 32.9 (CH_2), 32.3 (CH_2), 29.3 (CH_2), 28.7 (3 x CH_3), 23.0 (CH_2), 20.1 (CH_2). Two CO, one C_q, five aromatic CH, one CH and one CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{46}H_{54}N_4O_6Na$ $[M+Na]^+$, 781.3936; found, 781.3933. HPLC purity, 100%, t_R = 23.3 min, gradient A. $[\alpha]^{24}_D$ -19.8 (c = 0.8, MeOH).

*(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-aminium 2,2,2-trifluoroacetate (**25**)*

Compound **25** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC employing method A (t_R = 13.5 min) to afford compound **25** as TFA salt (163 mg, 0.215 mmol, 75%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.97-7.95 (m, 2H, $H_{25, 44, 48}$), 7.63-7.61 (m, 1H, H_{46}), 7.53-7.51 (m, 2H, $H_{45, 47}$), 7.31-7.16 (m, 15H, $H_{1\text{-}3, 6, 27, 33\text{-}42}$), 4.95 (br s, 1H, H_8), 4.85 (d, J = 15.8 Hz, 1H, H_{10a}), 4.61 (br s, 1H, H_{10b}), 4.25 (t, J = 7.8 Hz, 1H, H_{30}), 3.99-3.96 (m, 1H, H_{29a}), 3.68-3.51 (m,

2H, $H_{24, 29b}$), 3.32 (t, *J* = 6.5 Hz, 2H, H_{16}), 3.21 (dd, *J* = 4.0 Hz, *J* =15.7 Hz, 1H, H_{7a}), 3.10-2.81 (m, 5H + H_2O, $H_{7b, 15, 20}$), 1.50 (s, 2H, H_{23}), 1.22-1.19 (m, 2H, H_{21}), 1.06-1.03 (m, 2H, H_{22}). ^{13}C-NMR (150 MHz, DMSO-*d*$_6$, 350 K, rotamers were observed): δ (ppm) 199.1 (C=O), 171.7 (C=O), 170.1 (C=O), 168.4 (C=O), 142.4 and 142.3 (2 x C_q, rotamers), 137.0 (C_q), 132.9 (C_q), 128.6 (2 x CH), 128.4 (2 x CH), 128.3 (4 x CH), 127.8 and 127.7 (4 x CH, rotamers), 126.42 and 126.4 (2 x CH, rotamers), 126.2 (CH), 126.0 (CH), 52.3 (CH), 50.1 (CH), 43.4 (CH_2), 33.4 (CH_2), 30.7 (CH_2) , 28.5 (CH_2), 27.6 (CH_2), 21.2 (CH_2). Four C_q, one CH, three aromatic CH and three CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{40}H_{45}N_4O_4$ $[M+H]^+$, 645.3435; found, 645.3434. HPLC purity, 99%, t_R = 19.5 min, gradient A. $[\alpha]^{24}_D$ -22.5 (c = 0.67, MeOH).

*(S)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(5-oxo-5-phenylpentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-aminium 2,2,2-trifluoroacetate (**26**)*

Compound **26** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC employing method C to afford compound **26** as colorless TFA salt (14 mg, 0.018 mmol, 43%). ^{1}H-NMR (600 MHz, DMSO-*d*$_6$, 350 K): δ (ppm) 7.95 (d, *J* = 7.6 Hz, 2H, $H_{45, 49}$), 7.63-7.59 (m, 1H, H_{47}), 7.51 (t, *J* = 7.8 Hz, 2H, $H_{46, 48}$), 7.44-7.15 (m, 17H, $H_{1-3, 6, 21, 23}$), 6.55 (br s, 1H, H_{15}), 4.98-4.47 (m, 3H, $H_{8, 10}$), 4.22 (t, *J* = 7.9 Hz, 1H, H_{26}), 3.77-3.71 (m, 2H, H_{25}), 3.20-2.30 (several m, 9H + H_2O + DMSO, $H_{7, 16, 20, 39, 41}$), 1.94-1.92 (m, 2H, H_{40}), 1.34 (br s, 2H, H_{19}), 1.17 (m, 2H, H_{17}), 1.01 (br, 2H, H_{18}). ^{13}C-NMR (150 MHz, DMSO-*d*$_6$, 350 K): δ (ppm) 143.3 (2 x C_q), 137.6 (C_q), 133.4 (C_q), 131.1 (C_q), 129.2 (2 x CH), 128.9 (4 x CH), 128.4 (4 x CH), 128.3 (2 x CH), 126.8 (2 x CH), 50.8 (CH) 43.5 (CH_2), 38.1 (CH_2), 35.1 (CH_2), 32.8 (CH_2), 29.5 (CH_2), 22.9 (CH_2), 20.1 (CH_2). Four C=O, two CH, five aromatic CH and three CH_2 groups were not detected due to peak

broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{41}H_{47}N_4O_4$ $[M+H]^+$, 659.3592; found, 659.3598. HPLC purity, 99 %, t_R = 19.8 min, gradient A.

*4-{(S)-3-[((S)-5-((tert-butoxycarbonyl)amino)-6-((2,2-diphenylethyl)amino)-6-oxohexyl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-4-oxobutanoic acid (**27**)*

To solution of succinic anhydride (43 mg, 0.43 mmol) in 2.5 ml anhydrous DCM was added compound **5** (50 mg, 0.085 mmol) was added to the reaction mixture, then it was warmed up at room temperature and stirred for 18 h. A saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with DCM. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and evaporated in *vacuo*. The crude solid mixture was purified by preparative HPLC employing method D (t_R = 17.0 min) to afford compound **27** as a colorless solid (47 mg, 0.069 mmol, 81%). ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 8.16 (br s, 1H, H_{49}), 7.25-7.14 (m, 15H, $H_{1\text{-}3,\ 6,\ 21,\ 34\text{-}43}$), 5.98 (br s, 1H, $H_{12,\ 26}$), 4.90 (br s, 1H, H_8), 4.78 (d, J = 14.7 Hz, 1H, H_{10a}), 4.55 (br s, 1H, H_{10b}), 4.22 (t, J = 7.7 Hz, 1H, H_{23}), 3.80-3.76 (m, 2H, $H_{18,\ 22a}$), 3.71-3.66(m, 1H, H_{22b}), 3.20-2.73 (several m, 8H + H_2O + DMSO, $H_{7,\ 14,\ 45,\ 47}$), 1.36-1.27 (m, 11H, $H_{17a,\ 31\text{-}33}$), 1.22-1.21 (m, 1H, H_{17b}), 1.20-1.16 (m, 2H, H_{15}), 1.08-1.00 (m, 2H, H_{16}). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 172.7 (C=O), 171.0 (C=O), 154.0 (C=O), 141.7 (2 x C_q), 132.5 (C_q), 127.33 and 127.29 (4 x CH, rotamers), 126.89 and 126.87 (4 x CH, rotamers), 125.7 (CH), 125.3 (2 x CH), 125.0 (CH), 77.1 (C_q), 53.6 (CH), 51.5 (CH), 49.2 (CH), 42.3 (CH_2), 40.4 (CH_2), 37.4 (CH_2), 30.8 (CH_2), 28.2 (CH_2), 27.7 (CH_2), 27.2 (3 x CH_3), 21.4 (CH_2). Two C=O, one C_q, two aromatic CH and two CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{39}H_{48}N_4O_7Na$ $[M+Na]^+$, 707.3415; found, 707.3417. LC-MS (ESI) m/z calcd. for

$C_{39}H_{49}N_4O_7$ $[M+H]^+$, 685.8; found, 685.9. HPLC purity, 97 %, t_R = 21.0 min, gradient A. $[\alpha]^{25}_D$ -21.1 (c = 0.74, MeOH).

*(S)-3-{[(S)-5-ammonio-6-((2,2-diphenylethyl)amino)-6-oxohexyl]carbamoyl}-1,2,3,4-tetrahydroisoquinolin-2-ium 2,2,2-trifluoroacetate (**28**)*

Compound **28** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC (Method E) to afford compound **28** as colorless TFA salt (36 mg, 0.05 mmol, 32%). ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 8.10 (s, 2H), 7.33-7.20 (m, 14H), 4.30-4.27 (m, 3H), 4.03 (dd, *J* = 4.9 Hz, *J* = 10.7 Hz, 1H), 3.98 (m, 1H), 3.65 (t, *J* = 7.4 Hz, 1H), 3.30-3.26 (dd, *J* = 4.9 Hz, *J* = 16.7 Hz, 1H), 3.13-3.03 (m, 2H + H_2O), 1.59-1.55 (m, 2H), 1.40-1.36 (m, 2H), 1.20-1.15 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 168.9 (C=O), 142.9 and 142.8 (2 x C_q, rotamers) 136.0 (C_q), 129.4 (CH), 128.9 and 128.8 (4 x CH, rotamers), 128.34 and 128.26 (4 x CH, rotamers), 128.0 (2 x CH), 127.3 (CH), 126.9 (2 x CH), 54.9 (CH), 52.8 (CH), 50.6 (CH) , 43.9 (CH_2), 39.0 (CH_2), 31.2 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 21.8 (CH_2). Two C=O, one C_q, two aromatic CH and two CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{30}H_{37}N_4O_4$ $[M+H]^+$, 485.2911; found, 485.2907. LC-MS (ESI) *m/z* calcd. for $C_{30}H_{37}N_4O_4$ $[M+H]^+$, 485.3; found, 485.6. HPLC purity, 95 %, t_R = 9.2 min, gradient B.

*N6-[(benzyloxy)carbonyl]-N2-(tert-butoxycarbonyl)-D-lysine (**29**)*

To a 1M aqueous solution of NaOH (37 mLat rt were added Z-D-Lys(Boc)-OH (3.00 g, 12.2) and dioxane slowly until the suspension dissolves. Then benzyl chloroformate was added (2.70 g, 2.3 µL, 15.9) dropwise over a period of 30 min at 0 °C. The reaction mixture was stirred for 16 h at room temperature. Furthermore dioxane was removed *in vacuo* and

reaction mixture was acidified to pH 2 with 1M HCl at 0 °C. The aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford compound **29** (4.4, 11.6 mmol, 95%) as a colorless foam. No further purification was required, No further purification was required, however sample was purified by flash column chromatography for the analysis. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 8.90 (br s, 1H), 7.37-7.23 (m, 5H), 6.18 (br s, 1H), 5.17-4.86 (m, 3H), 4.83 (s, 1H), 4.22-4.04 (m, 1H), 3.13-3.11 (m, 2H), 1.76-1.37 (m, 15H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 176.4 (C=O), 156.8 (C=O), 156.0 (C=O), 136.6 (C_q), 128.7 (2 x CH), 128.3 (2 x CH), 128.1 (CH), 80.4 (C_q) 66.9 (CH_2), 53.3 (CH), 40.7 (CH_2), 32.0 (CH_2), 29.3 (CH_2), 29.5 (3 x CH_3), 22.1 (CH_2). MS (EI) *m/z* (%): 280 (2, $[M + H]^+$ - $C_5H_9O_2$), 222 (14), ($C_7H_{12}NO_4$) 174 (12, M^+ - $C_{12}H_{16}NO_2$), 128 (32), 108 (27), 92 (11), (C_7H_7) 91 (100), 84 (20), 57 (52), 41 (20). MS (EI) m/z calcd for $C_{14}H_{20}N_2O_4$ $[M + H - C_5H_9O_2]^+$, 280.1; found 280.1. LC-MS (ESI) *m/z* calcd. for $C_{19}H_{28}N_2O_6Na$ $[M+Na]^+$, 403.2; found, 403.7. HPLC purity, 96%, t_R = 19.7 min, gradient A.

*Benzyl tert-butyl {6-[(2,2-diphenylethyl)amino]-6-oxohexane-1,5-diyl}(R)-dicarbamate (**30**)*

Compound **30** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 98/2) to afford compound **30** (3.00 g, 9.60, 5.36mmol, 80%) as a colorless solid. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 7.28-7.21 (m, 9H), 7.16-7.13 (m, 6H), 5.94 (s, 1H), 5.02 (m, 2H), 4.92 (s, 1H), 4.70 (s, 1H), 4.10 (t, *J* = 8.0 Hz, 1H), 3.89-3.74 (m, 3H), 3.05-3.02 (m, 2H), 1.61-1.58 (m, 1H), 1.40-1.34 (m, 3H), 1.31 (s, 9H), 1.14-1.12 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 170.8 (C=O), 155.5 (C=O), 154.6 (C=O), 140.7 and 140.6 (2 x C_q, rotamers), 135.6 (C_q), 127.72 and 127.69 (4 x CH, rotamers), 127.5 (2 x CH), 127.1 (2 x CH), 127.0 (4 x CH), 125.9 (2 x CH), 79.0 (C_q), 65.6 (CH_2), 53.23 (CH), 49.6 (CH), 42.7 (CH_2), 39.4 (CH_2), 31.0 (CH_2), 28.4 (CH_2), 27.3 (3 x CH_3), 21.3 (CH_2). MS (EI) m/z (%): 559 (1, M^+), 307 (26), 263 (11), 218

(18), 180 (34), 174 (15), 167 (23), 108 (18), 107 (14), 91 (100), 84 (21), 79 (19), 77 (13), 59 (15), 57 (48), 41 (22), 30 (38). MS (EI) m/z calcd for $C_{33}H_{41}N_3O_5$ $[M]^+$ 559.3; found, 559.3. LC-MS (ESI) m/z calcd. for $C_{33}H_{41}N_3O_5Na$ $[M+Na]^+$, 582.7; found, 582.4. HPLC purity, 100%, t_R = 13.2 min, gradient B. $[\alpha]^{25}_D$ +20.1 (c = 0.66, MeOH).

*tert-Butyl (R)-{6-amino-1-[(2,2-diphenylethyl)amino]-1-oxohexan-2-yl}carbamate (**31**)*

Compound **31** was prepared according to general procedure 3 (**GP3**), to afford the titled compound **31** (2.21 g, 5.18 mmol, 98%) as a white solid. ^{1}H-NMR (360 MHz, $CDCl_3$): δ (ppm) 7.25-7.21 (m, 4H), 7.17-7.14 (m, 6H), 6.23 (s, 1H), 4.94 (s, 1H), 4.11 (t, *J* = 8.0 Hz, 1H), 3.85-3.74 (m, 3H), 2.57-2.51 (m, 2H), 1.63-1.57 (m, 1H), 1.44-1.13 (m, 15H). ^{13}C-NMR (90 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.9 (C=O), 154.5 (C=O), 140.8 (C_q), 140.7 (C_q), 127.71 and 127.68 (4 × CH, rotamers), 127.0 (4 x CH), 125.8 (2 × CH), 78.9 (C_q), 53.3 (CH), 49.6 (CH), 42.7 (CH_2), 40.6 (CH_2), 31.8 (2 x CH_2), 27.3 (3 × CH_3), 21.6 (CH_2). MS (EI) m/z (%): 425 (12, M^+), 258 (14, M^+- $C_{13}H_{11}$), 202 (18), 181 (11), 180 (25), 173 (14), 167 (27), 165 (18), 145 (13), 139 (15), 129 (12), 84 (100), 82 (18), 57 (50), 56 (18), 41 (26), 30 (36). MS (EI) m/z calcd for $C_{25}H_{35}N_3O_3$ $[M]^+$, 425.3, found 425.3. LC-MS (ESI) m/z calcd. for $C_{25}H_{36}N_3O_3$ $[M+H]^+$, 426.3; found, 426.8. HPLC purity, 96%, t_R = 17.6 min, gradient A. $[\alpha]^{25}_D$ +25.3 (c = 0.93, MeOH).

*Benzyl (S)-3-{[(R)-5-((tert-butoxycarbonyl)amino)-6-((2,2-diphenylethyl)amino)-6-oxohexyl] carbamoyl}-3,4-dihydroisoquinoline-2(1H)-carboxylate (**32**)*

Compound **32** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 9/3/1) to afford compound **32** (0.60 g, 0.84 mmol, 70%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.42-7.18 (several m, 19H), 6.03 (br s, 1H), 5.20 (q, *J* = 9.3 Hz, 1H), 4.77-4.72 (m,

2H), 4.58 (d, *J* = 15.8 Hz, 1H), 4.27 (t, *J* = 8.0 Hz, 1H), 3.86-3.80 (m, 2H), 3.76-3.71 (m, 1H), 3.15 (d, *J* = 5.5 Hz, 2H), 2.95-2.91 (m, 2H + H_2O), 1.47-1.43 (m, 1H), 1.41 (s, 9H), 1.37-1.32 (m, 1H), 1.26-1.21 (2H), 1.12-1.05 (2H). ^{13}C-NMR (90 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.9 (C=O), 168.1 (C=O), 154.5 (C=O), 140.7 (2 x C_q), 135.1 (C_q), 132.6 (C_q), 127.71 and 127.68 (4 x CH, rotamers), 127.6 (2 x CH), 127.3 (CH), 127.05 and 127.02 (4 x CH, rotamers), 126.7 (CH), 125.8 (2 x CH), 78.9 (C_q), 66.8 (CH_2), 53.4 (CH), 49.6 (CH), 44.1 (CH_2), 42.7 (CH_2), 37.5 (CH_2), 30.6 (2 x CH_2), 27.9 (CH_2), 27.3 (3 x CH_3), 21.1 (CH_2). One C=O, one C_q, five aromatic CH and one CH carbons was not detected due to peak broadening and overlapping. LC-MS (ESI) m/z calcd. for $C_{43}H_{51}N_4O_6Na$ $[M+H]^+$, 741.4; found, 741.2. HPLC purity, 100%, t_R = 22.4 min, gradient A. $[\alpha]^{22}_D$ +4.3 (c = 0.70, MeOH).

*tert-Butyl {(R)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**33**)*

Compound **33** was prepared according to general procedure 3 (**GP3**), to afford compound **33** (0.40 g, 0.68 mmol, 97%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.24-7.19 (m, 4H), 7.17-7.08 (m, 10H), 6.97-6.96 (m, 1H), 6.06 (br s, 1H), 4.97 (s, 1H), 4.12 (t, *J* = 8.0 Hz, 1H), 3.95-3.74 (m, 5H), 3.45 (dd, *J* = 5.1 Hz, *J* = 10.6 Hz, 1H), 3.17-3.12 (m, 3H), 2.74 (dd, *J* = 10.7 Hz, *J* = 16 Hz, 1H), 1.62-1.61 (m, 1H + H_2O), 1.41-1.35 (m, 3H), 1.31 (s, 9H), 1.20-1.12 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 172.2 (C=O), 170.9 (C=O), 154.6 (C=O), 140.7 (2 x C_q), 134.8 (C_q), 133.3 (C_q), 128.2 (CH), 127.73 and 127.67 (4 x CH, rotamers), 127.0 (4 x CH), 125.8 (2 x CH), 125.6 (CH), 125.1 (CH), 124.5 (CH), 78.9 (C_q), 55.5 (CH), 53.4 (CH), 49.6 (CH_2), 46.6 (CH), 42.7 (CH_2), 37.2 (CH_2), 30.8 (CH_2), 30.0 (CH_2), 28.1 (CH_2), 27.3 (3 x CH_3), 21.4 (CH_2). MS (EI) *m/z* (%): 133 (14, $[M+H]^+$ - $C_{26}H_{34}N_3O_4$), 132 (100, M^+ - $C_{26}H_{34}N_3O_4$), 131 (20), 130 (11), 84 (11), 59

(32), 57 (12), 41 (17). MS (EI) m/z calcd for $C_{35}H_{44}N_4O_4$ $[M]^+$ 584.3, found 584.3. LC-MS (ESI) *m/z* calcd. for $C_{35}H_{45}N_4O_4$ $[M+H]^+$, 585.3; found, 585.9. HPLC purity, 91%, t_R = 20.5 min, gradient A. $[\alpha]^{25}_D$ -16.8 (c = 0.83, MeOH).

*tert-Butyl {(R)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-yl}carbamate (**34**)*

Compound **34** was prepared according to general procedure 2 (**GP2**) by using 3-benzoylpropionic acid**.** The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 9/3/1) to afford compound **34** (93 mg, 0.13 mmol, 73%) as a colorless solid. ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.97-7.95 (m, 2H, $H_{44, 48}$), 7.63-7.60 (m, 1H, H_{46}), 7.53-7.50 (m, 2H, $H_{45, 47}$), 7.28-7.16 (m, 15H, $H_{1\text{-}3, 6, 27, 33\text{-}42}$), 5.99 (br s, 1H, $H_{19, 25}$), 4.94 (br s, 1H, H_8), 4.83 (d, J = 15.9 Hz, 1H, H_{10a}), 4.60 (br s, 1H, H_{10b}), 4.22 (t, J = 7.7 Hz, 1H, H_{30}), 3.83-3.76 (m, 2H, $H_{24, 29a}$), 3.71-3.67 (m, 1H, H_{29b}), 3.31 (t, J = 6.4 Hz, 2H, H_{16}), 3.21 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.08 (br s, 1H, H_{7b}), 2.95-2.76 (m, 4H + H_2O, $H_{15, 20}$), 1.43-1.37 (m, 1H, H_{23a}), 1.36 (s, 9H, $H_{53\text{-}55}$), 1.35-1.27 (m, 1H, H_{23b}), 1.22-1.19 (m, 2H, H_{21}), 1.08-1.01 (m, 2H, H_{22}). ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.92-7.91 (m, 2H), 7.52-7.51 (m, 1H,), 7.43-7.40 (m, 2H), 7.21-7.10 (m, 15H), 6.64-6.57 (m, 1H), 6.05-5.95 (br s, 1H), 5.25 (br s, 1H), 5.03-4.57 (several m, 3H), 4.10-4.03 (m, 1H), 3.90-3.63 (several m, 4H), 3.48-3.42 (m, 1H), 3.25-2.73 (several m, 5H), 2.54-2.47 (m, 1H), 1.49-1.46 (m, 1H + H_2O), 1.35-1.34 (m, 1H), 1.32 (s, 9H), 1.28-0.93 (several m, 4H). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): 171.5 (C=O), 142.3 (2 x C_q), 136.6 (C_q), 132.5 (C_q), 127.88-127.84 (4 x CH, rotamers), 127.44 and 128.42 (4 x CH, rotamers), 127.4 (CH), 125.8 (2 x CH), 125.5 (CH), 77.6 (C_q), 49.7 (CH), 45.0 (CH_2), 42.8 (CH_2), 33.0 (CH_2), 31.3 (CH_2), 28.2 (CH_2), 27.7 (3 x CH_3), 27.2 (CH_2), 23.0 (CH_2). Four C=O, One C_q, two CH, four aromatic CH and two CH_2 groups were not detected due to peak broadening or

overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{45}H_{52}N_4O_6Na$ $[M+Na]^+$, 767.3779; found, 767.3774. HPLC purity, 98%, t_R = 21.7 min, gradient A. $[\alpha]^{22}_D$ -0.2 (c = 1.2, MeOH).

*(R)-1-[(2,2-diphenylethyl)amino]-1-oxo-6-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-2-aminium 2,2,2-trifluoroacetate (**35**)*

Compound **35** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC employing method C (t_R = 16.1 min) to afford compound **35** as colorless TFA salt (45 mg, 0.059 mmol, 73%). ^{1}H-NMR (600 MHz, DMSO-d_6, 400 K): δ (ppm) 7.97-7.95 (m, 2H, $H_{34, 38}$), 7.63-7.61 (m, 1H, H_{36}), 7.53-7.51 (m, 2H, $H_{35, 37}$), 7.31-7.16 (m, 15H, $H_{1-3, 6, 29, 39-48}$), 4.95 (br s, 1H, H_8), 4.84 (d, J = 15.8 Hz, 1H, H_{10a}), 4.60 (br s, 1H, H_{10b}), 4.25 (t, J = 7.8 Hz, 1H, H_{31}), 3.97 (br s, 1H, H_{30a}), 3.67-3.64 (m, 1H, H_{30b}), 3.32 (t, J = 6.5 Hz, 2H, H_{14}), 3.21 (dd, J = 4.0 Hz, J =15.7 Hz, 1H, H_{7a}), 3.10-2.81 (m, 6H + H_2O, $H_{7b, 13, 21, 25}$), 1.51 (s, 2H, H_{24}), 1.22-1.18 (m, 2H, H_{22}), 1.05-1.03 (m, 2H, H_{23}). ^{13}C-NMR (150 MHz, DMSO-d_6, rotamers were observed): δ (ppm) 199.1 (C=O), 171.5 and 171.3 (C=O, rotamers), 170.07 and 169.85 (C=O, rotamers), 168.4 (CO), 142.4 and 142.3 (2 x C_q, rotamers), 136.65 and 136.63 (C_q, rotamers), 132.4 (C_q), 133.1 (C_q), 128.7 (2 x CH), 128.5 (2 x CH), 128.4 (4 x CH), 127.8 (4 x CH), 127.7 (2 x CH), 126.7 (CH), 126.5 (2 x CH), 126.3 (CH), 126.0 (CH), 55.1 (CH), 52.4 (CH), 50.1 (CH), 45.2 (CH_2), 43.1 (CH_2), 33.2 (CH_2), 31.3 (CH_2), 30.7 (CH_2) , 28.5 (CH_2), 27.6 (CH_2), 21.0 (CH_2). One CH_2 group was not detected due to peak broadening or overlapping caused by the high temperature. HRMS (ESI) m/z calcd for $C_{40}H_{45}N_4O_4$ $[M+H]^+$, 645.3435; found, 645.3433. HPLC purity, 99%, t_R = 18.2 min, gradient A.

*Benzyl tert-butyl {6-[(2,2-diphenylethyl)amino]-6-oxohexane-1,5-diyl}(S)-dicarbamate (**36**)*[28]

Compound **36** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **36** (1.84 g, 3.3 mmol, 97%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.40-7.06 (m, 15H + $CDCl_3$), 5.93 (br s, 1H), 5.25-5.22 (m, 1H), 4.98-4.93 (m, 2H), 4.45 (br s, 1H), 4.10 (t, *J* = 7.8 Hz, 1H), 3.89-3.73 (m, 3H), 2.95 (br s, 2H) 1.62 (br s, 1H), 1.41-1.11 (m, 14H).). MS (EI) m/z (%): 181 (18, $C_{14}H_{13}$), 180 (78), 168 (16, $C_{13}H_{12}$), 166 (12, $C_{13}H_{10}$), 165 (19), 152 (12), 128 (10.1), 91 (100), 84 (21), 82 (12), 57 (13), 30 (13). MS (EI) m/z calcd for $C_{33}H_{41}N_3O_5$ $[M]^+$ 559.3 , found 559.3.

*Benzyl tert-butyl {6-[(2,2-diphenylethyl)amino]-6-oxohexane-1,5-diyl}(R)-dicarbamate (**37**)*

Compound **37** was prepared according to general procedure 1 (**GP1**). The crude solid was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **37** (2.9mg, 5.2 mmol, 99%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.28-7.13 (m, 15H), 5.96 (br s, 1H), 5.27 (br s, 1H), 4.98-4.93 (m, 2H), 4.46 (br s, 1H), 4.09 (t, J = 7.8 Hz, 1H), 3.89-3.73 (m, 3H), 2.95 (br s, 2H) 1.62-1.59 (m, 1H), 1.35-1.09 (m, 14H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.7 (C=O), 155.1 (C=O), 140.6 (2 x C_q), 135.1 (C_q), 127.71 and 127.69 (4 x CH, rotamers), 127.5 (2 x CH), 127.2 (CH), 127.1 (2 x CH), 127.05 and 127.99 (4x CH, rotamers), 125.8 (2 x CH), 78.2 (C_q), 66.0 (CH_2), 53.8 (CH), 49.5 (CH), 42.7 (CH_2), 38.7 (CH_2), 30.9 (CH_2), 28.6 (CH_2), 27.4 (3 x CH_3), 21.2 (CH_2). HPLC purity, 100%, t_R = 21.6 min, gradient A. $[\alpha]^{24}_D$ +22.0 (c = 0.77, MeOH).

*tert-Butyl (S)-{5-amino-6-[(2,2-diphenylethyl)amino]-6-oxohexyl}carbamate (**38**)*[28]

Compound **38** was prepared according to general procedure 3 (**GP3**), to afford compound **38** (1.06 g, 2.49 mmol, 93%) as a yellow oil. No further purification was required for the next step. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.25-7.22 (m, 4H), 7.19-7.13 (m, 7H), 4.45 (s,

1H), 4.13 (t, *J* = 8.0 Hz, 1H), 3.86 (ddd, *J* = 6.2 Hz, *J* = 8.1 Hz, *J* = 14.2 Hz, 1H), 3.79 (ddd, *J* = 6.2 Hz, *J* = 8.1 Hz, *J* = 14.2 Hz, 1H), 3.19 (dd, *J* = 4.6 Hz, *J* = 7.8 Hz, 1H), 3.00-2.99 (m, 2H), 1.62-1.58 (m, 1H), 1.37-1.32 (m, 12H), 1.19-1.14 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 175.2 (C=O), 156.4 (C=O), 142.33-142.31 (d, 2 x C_q) 129.99-128.97 (4 x CH, rotamers), 128.44 and 128.40 (4 x CH, rotamers), 127.1 (2 x CH), 77.6 (C_q), 55.3 (CH), 51.1 (CH), 43.7 (CH_2), 40.5 (CH_2), 34.9 (CH_2), 31.5 (CH_2), 30.2 (CH_2), 28.8 (3 x CH_3), 23.0 (CH_2).). MS (EI) m/z (%): 201 (53, $C_{10}H_{21}N_2O_2$), 180 (16, $C_{14}H_{12}$), 167 (16, $C_{13}H_{11}$), 165 (12), 158 (11), 145 (30), 139 (12), 128 (36), 127 (18), 101 (11), 84 (100), 82 (12), 57 (31), 56 (21), 44 (10), 43 (11), 41 (22), 30 (15). MS (EI) m/z calcd for $C_{25}H_{35}N_3O_3$ $[M]^+$ 425.3; found, 425.3. LC-MS (ESI) m/z calcd. for $C_{25}H_{37}N_3O_3$ $[M+H]^+$, 426.3; found, 426.3, t_R = 11.2 min, gradient B.

*tert-Butyl (R)-{5-amino-6-[(2,2-diphenylethyl)amino]-6-oxohexyl}carbamate (**39**)*

Compound **39** was prepared according to general procedure 3 (**GP3**), to afford compound **39** (2.217 g, 5.10 mmol, 99%) as a yellow oil. No further purification was required for the next step. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.24-7.22 (m, 4H), 7.19-7.13 (m, 7H), 4.46 (s, 1H), 4.14 (t, *J* = 8.0 Hz, 1H), 3.83(ddd, *J* = 6.2 Hz, *J* = 8.1 Hz, *J* = 14.3 Hz, 1H), 3.78 (ddd, *J* = 6.2 Hz, *J* = 8.1 Hz, *J* = 14.3 Hz, 1H), 3.17 (dd, *J* = 4.5 Hz, *J* = 7.8 Hz, 1H), 3.00-2.99 (m, 2H), 1.62-1.58 (m, 1H), 1.37-1.30 (m, 12H), 1.19-1.14 (m, 2H). ^{13}C-NMR (90 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 173.8 (C=O), 155.0 (C=O), 140.96 and 140.94 (2 x C_q, rotamers), 127.62-127.61 (4 x CH, rotamers), 127.07-127.03 (4 x CH), 125.7 (2 x CH), 78.1 (C_q), 54.0 (CH), 49.7 (CH), 42.3 (CH_2), 39.2 (CH_2), 33.6 (2 x CH_2), 27.4 (3 x CH_3), 21.6 (CH_2). MS (EI) *m/z* (%): 201 (54, $C_{10}H_{21}N_2O_2$), 180 (16, $C_{14}H_{12}$), 167 (16, $C_{14}H_{12}$), 165 (13), 158 (11), 145 (34), 139 (15), 128 (36), 127 (11), 84 (100), 82 (14), 57 (34), 56 (20), 44 (12), 43 (11), 41 (24), 30 (16). MS (EI) m/z calcd for $C_{25}H_{35}N_3O_3$ $[M]^+$, 425.3; found, 425.3. LC-

MS (ESI) *m/z* calcd. for $C_{25}H_{36}N_3O_3$ $[M+H]^+$, 426.6; found, 426.9. HPLC purity, 98%, t_R = 17.5 min, gradient A. $[\alpha]^{24}_D$ +2.8 (c = 1.0, MeOH).

*Benzyl (S)-3-{[(S)-6-((tert-butoxycarbonyl)amino)-1-((2,2-diphenylethyl)amino)-1-oxohexan-2-yl]carbamoyl}-3,4-dihydroisoquinoline-2(1H)-carboxylate (**40**)*

Compound **40** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **40** (1.01 g, 1.41 mmol, 48%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 7.30-7.09 (m, 19H + $CDCl_3$), 6.24 (d, *J* = 8.0 Hz, 1H), 6.06 and 5.76 (s, 1H, rotamers), 5.18-5.09 (m, 2H), 4.66-4.37 (m, 4H), 4.11-3.91 (m, 2H), 3.80 (ddd, *J* = 6.4 Hz, *J* = 8.0 Hz, *J* = 14.2 Hz, 1H), 3.71 and 3.51 (br s, 1H, rotamers) 3.19-3.16 (m, 1H), 2.95 (br s, 1H), 2.81 (br s, 2H), 1.48-0.99 (m, 15H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 170.1 (C=O), 155.9 (2 x C=O), 140.8 (2 x C_q), 140.0 (C_q), 135.2 (C_q), 132.1 (C_q), 127.84 and 127.81 (4 x CH, rotamers), 127.5 (CH), 127.3 (2 x CH), 127.0, 126.3, 126.0, 125.2, 78.3 (C_q), 67.2 (CH), 64.5 (CH_2) 55.2 (CH), 51.6 (CH), 49.6 (CH), 44.4 (CH_2), 42.9 (CH_2), 39.1 (CH_2), 30.7 (CH_2), 28.4 (CH_2), 27.6 (3 x CH_3), 21.1 (CH_2). HPLC purity, 93%, t_R = 20.5 min, gradient A.

*Benzyl (S)-3-{[(R)-6-((tert-butoxycarbonyl)amino)-1-((2,2-diphenylethyl)amino)-1-oxohexan-2-yl]carbamoyl}-3,4-dihydroisoquinoline-2(1H)-carboxylate (**41**)*

Compound **41** was prepared according to general procedure 1 (**GP1**). The crude solid mixture was purified by flash column chromatography (DCM/MeOH: 96/4) to afford compound **41** (0.45 g, 0.62 mmol, 53%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.30-7.07 (m, 19H + $CHCl_3$), 6.30-6.05 (m, 1H), 6.05 (br s, rotamer 1) and 5.45 (br s, rotamer 2, 1H), 5.14 (br s, 2H), 4.66-4.39 (m, 4H), 4.05 (br s, 2H), 3.67 (br s, 2H), 3.12-2.88 (m, 4H),

1.36-0.81 (m, 15H). ^{13}C-NMR (90 MHz, $CDCl_3$): δ (ppm) 171.3 (C=O), 156.4 (C=O), 142.1 (C_q), 141.8 (C_q), 136.4 (C_q) 133.8 (C_q), 129.0 (4 x CH), 128.9 (CH), 128.6 (CH), 128.4 (CH), 128.3 (CH), 128.0 (2 x CH), 127.9 (6 x CH), 127.3 (CH), 127.2 (CH), 126.4 (CH), 79.5 (C_q), 68.2 (CH_2), 65.7 (CH), 56.2 (CH), 53.2 (CH), 50.7 (CH_2), 45.3(CH_2), 44.2 (CH_2), 40.4 (CH_2), 30.6 (CH_2), 29.7 (CH_2), 28.8 (3 x CH_3), 22.5 (CH_2). HPLC purity, 94%, t_R = 23.0 min, gradient A.

*tert-Butyl {(S)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexyl}carbamate (**42**)*

Compound **42** was prepared according to general procedure 3 (**GP3**), to afford compound **42** (0.81 g, 1.38 mmol, 100%) as a colorless solid. No further purification was required. ^{1}H-NMR (600 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 7.44 (d, *J* = 8.4 Hz, 1H), 7.19-7.06 (m, 13H), 6.99-6.97 (m, 1H), 6.18 (t, *J* = 5.7 Hz, 1H), 4.47 (br s, 1H), 4.15-4.11 (m, 2H), 3.91-3.77 (m, 4H), 3.27 (dd, *J* = 5.3 Hz, *J* = 9.9 Hz, 1H), 3.04 and 3.01 (2 x d, each *J* = 5.3 Hz, 2H), 2.93 (br s, 2H), 2.69 (dd, *J* = 9.9 Hz, J = 16.1 Hz, 1H), 1.68-1.64 (m, 1H), 1.42-1.28 (m, 12H), 1.05 (br s, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 172.5 (C=O), 170.3 (C=O), 155.0 (C=O), 140.7 (C_q), 140.6 (C_q), 134.8 (C_q), 133.0 (C_q), 128.0 (CH), 127.7 (4 x CH), 127.0 (4 x CH), 125.8 (2 x CH), 125.7 (CH), 125.3 (CH), 124.6 (CH), 78.1 (C_q), 55.1 (CH), 51.5 (CH), 49.6 (CH), 46.1 (CH_2), 42.6 (CH_2), 39.0 (CH_2), 30.3 (CH_2), 29.9 (CH_2), 28.5 (CH_2), 27.4 (3 x CH_3), 21.6 (CH_2). LC-MS (ESI) m/z calcd. for $C_{35}H_{45}N_4O_4$ $[M+H]^+$, 585.3; found, 585.9 $[M+H]^+$. HPLC purity, 96%, t_R = 21 min, gradient A. $[\alpha]^{24}_D$ -46.9 (c = 0.80, MeOH).

*tert-Butyl {(R)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexyl}carbamate (**43**)*

Compound **43** was prepared according to general procedure 3 (**GP3**), to afford the titled compound **43** (351 mg, 0.604 mmol, 97%) colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$, , rotamers were observed): δ (ppm) 7.45 (d, *J* = 8.5 Hz, 1H), 7.17-7.05 (m, 14H + $CDCl_3$), 6.97 (m, 1H), 6.10 (br s, 1H), 4.48 (br s, 1H), 4.19 (dd, *J* = 8.0 Hz, *J* = 14.2 Hz, 1H), 4.11 (t, *J* = 8.0 Hz, 1H), 3.89 and 3.87 (s, 1H, rotamers), 3.81-3.78 (m, 3H), 3.40 (dd, *J* = 5.2 Hz, *J* = 10.6 Hz, 1H), 3.02-2.97 (m, 3H), 2.49 (dd, *J* = 10.6 Hz, *J* = 16.3 Hz, 1H), 1.74-1.70 (m, 1H), 1.48-1.45 (m, 1H), 1.36 (br s, 12H), 1.18-1.13 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 172.3 (C=O), 170.3 (C=O), 155.0 (C=O), 140.63 and 14.60 (2 x C_q, rotamers) 134.9 (C_q), 133.1 (C_q), 128.1 (CH), 127.7 (4 x CH), 127.01 and 126.99 (4 x CH, rotamers), 125.8 (2 x CH), 125.6 (CH) and 125.3 (CH), 124.6 (CH), 78.1 (C_q), 55.1 (CH), 51.6 (CH), 49.6 (CH), 46.5 (CH_2), 42.5 (CH_2), 39.1 (CH_2), 30.3 (CH_2), 29.5 (CH_2), 28.6 (CH_2), 27.4 (3 x CH_3), 21.7 (CH_2). LC-MS (ESI) m/z calcd. for $C_{35}H_{45}N_4O_4$ $[M+H]^+$, 585.3; found, 586.1. HPLC purity, 94%, t_R = 18.4 min, gradient A. $[\alpha]^{24}_D$ -14.7 (c = 0.61, MeOH).

*tert-Butyl {(S)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexyl}carbamate (**44**)*[28]

Compound **44** was prepared according to general procedure 2 (**GP2**) by using 3-benzoylpropionic acid. The crude solid mixture was purified by flash column chromatography (Hex/EtOAc/EtOH: 4/3/1) to afford compound **44** (237 mg, 0.30 mmol, 94%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$, rotamers were observed): δ (ppm) 7.93-7.84 (m, 2H), 7.54-7.49 (m, 1H), 7.43-7.37 (m, 2H), 7.20-6.98 (m, 15H + $CDCl_3$), 6.64 and 6.49 (2 x d, each *J* = 8.0 Hz, 1H, rotamers), 6.32 and 6.25 (2 x t, each *J* = 5.4 Hz, 1H, rotamers), 4.92-4.86 (m, 2H), 4.67-4.38 (m, 2H) , 4.03-3.82 (m, 2H), 3.64-3.52 (m, 2H), 3.33-3.18 (m, 2H), 3.07-2.81 (m, 4H), 2.50-2.36 (m, 1H), 1.73-1.67 (m, 1H), 1.44-1.32 (m,

10H), 1.19-1.07 (m, 2H), 0.81-0.58 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 199.5 (C=O), 172.7 (C=O), 169.4 (C=O), 154.9 (C=O), 141.0 (2 x C_q), 134.9 (C_q) 133.1 (C_q), 132.8 (C_q), 127.8, 127.7, 127.5, 127.4, 127.2, 127.1, 127.0, 126.9, 126.4, 126.3, 126.1, 125.6, 125.5, 125.4, 125.0, 78.0, 55.8, 53.6, 52.7, 52.0, 49.2, 45.7, 43.4, 42.8, 33.6, 28.1, 27.5 (3 x CH_3), 26.2, 21.6. Several peaks could not be assigned, due to the presence of rotamers. HRMS (ESI) m/z calcd for $C_{45}H_{52}N_4O_6Na$ $[M+Na]^+$, 767.3779; found, 767.3792. HPLC purity, 98%, t_R = 21.9 min, gradient A. $[\alpha]^{24}_D$ -17.0 (c = 0.60, MeOH).

*tert-Butyl {(R)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexyl}carbamate (**45**)*

Compound **45** was prepared according to general procedure 2 (**GP2**) by using 3-benzoylpropionic acid**.** The crude solid mixture was purified twice by flash column chromatography (Hex/EtOAc/EtOH: 7/3/1) to afford compound **45** (90 mg, 0.12 mmol, 71%) as a colorless solid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 7.94-7.90 (m, 2H), 7.52 (m, 1H), 7.42-7.38 (m, 2H), 7.19-7.06 (m, 14H + $CDCl_3$), 6.99 (d, *J* = 7.4 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H), 5.96 (br s, 1H), 5.09 (t, *J* = 5.0 Hz, 1H), 4.76-4.40 (m, 2H) , 4.12-3.96 (m, 2H), 3.64-3.47 (m, 2H), 3.04-2.66 (m, 4H), 2.56-2.51 (m, 1H), 1.71-1.66 (m, 1H), 1.39-1.32 (m, 10H), 1.27-1.14 (m, 2H), 1.05-0.90 (m, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 199.5 (C=O), 172.7 (C=O), 169.4 (C=O), 154.9 (C=O), 141.0 (2 x C_q), 134.9 (C_q) 133.1 (C_q), 132.8 (C_q), 127.8, 127.7, 127.5, 127.4, 127.2, 127.1, 127.0, 126.9, 126.4, 126.3, 126.1, 125.6, 125.5, 125.4, 125.0, 78.0 (C_q), 55.8 (CH), 53.6 (CH), 52.0 (CH), 49.2 (CH_2), 45.7 (CH_2), 43.4 (CH_2), 42.8 (CH_2), 33.6 (CH_2), 29.8 (CH_2), 29.4 (CH_2), 27.5 (3 x CH_3), 26.2 (CH_2), 21.6 (CH_2). Several peaks could not be assigned, due to the presence of rotamers. HRMS (ESI) m/z calcd for $C_{45}H_{52}N_4O_6Na$ $[M+Na]^+$, 767.3779; found, 767.3781. LC- HPLC purity, 98 %, t_R = 21.8 min, gradient A.

*(S)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-1-aminium 2,2,2-trifluoroacetate **(46)***[28]

Compound **46** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC employing method B (t_R = 12 min) to afford compound **46** as a colorless TFA salt (35 mg, 0.047 mmol, 69 %). ^{1}H-NMR (600 MHz, DMSO-d_6, 400K): δ (ppm) 8.01-7.99 (m, 2H), 7.65-7.64 (m, 1H), 7.56-7.54 (m, 4H), 7.32-7.19 (m, 15H), 4.94 (t, *J* = 5.2 Hz, 1H), 4.84 (d, *J* = 15.7 Hz, 1H), 4.65 (br s, 1H), 4.22 (t, *J* = 7.7 Hz, 1H), 4.09 (dd, *J* = 7.9 Hz, *J* = 13.7 Hz, 1H), 3.80 (ddd, *J* = 5.4 Hz, *J* = 7.5 Hz, *J* = 13.1 Hz, 1H), 3.65 (ddd, *J* = 6.2 Hz, *J* = 7.9 Hz, *J* = 13.8 Hz, 1H), 3.35 (t, *J* = 6.5 Hz, 2H), 3.15 (br s, 2H), 2.83 (br s, 2H), 2.70 (m, 2H), 1.53-1.47 (m, 1H), 1.45-1.40 (m, 2H), 1.37-1.31 (m, 1H), 1.05-0.97 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6, 350 K, rotamers were observed): δ (ppm) 199.5 (C=O), 171.5 (C=O), 143.1 and 143.0 (2 x C_q, rotamers) 137.4 (C_q), 133.4 (C_q), 129.2 (CH), 128.9 (2 x CH), 128.76 and 128.72 (4 x CH, rotamers), 128.32 (2 x CH), 128.26 and 128.24 (4 x CH), 128.1 (CH),) 126.6 (2 x CH), 50.5 (CH), 43.7 (CH_2), 39.3 (CH_2), 33.9 (CH_2), 31.5 (CH_2), 28.1 (CH_2), 27.0 (CH_2), 22.1 (CH_2). Three C=O, one C_q, two aromatic CH, two CH and two CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature.. HRMS (ESI) m/z calcd for $C_{40}H_{45}N_4O_4$ $[M+H]^+$, 645.3435; found, 645.3434. HPLC purity, 100 %, t_R = 18 min, gradient A.

*(R)-6-[(2,2-diphenylethyl)amino]-6-oxo-5-[(S)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido]hexan-1-aminium 2,2,2-trifluoroacetate (**47**)*

Compound **47** was prepared according to general procedure 4 (**GP4**). The crude solid mixture was purified by preparative HPLC employing method C (t_R = 15.3 min) to afford compound **47** as a colorless TFA salt (25 mg, 0.038 mmol, 76%). ^{1}H-NMR (600 MHz, DMSO-d_6, 400

K): δ (ppm) 7.94-7.93(m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.49-7.47 (m, 4H), 7.34 (m, 1H), 7.25-7.14 (m, 15H), 4.91 (t, *J* = 5.2 Hz, 1H), 4.82 (d, *J* = 15.7 Hz, 1H), 4.53 (br s, 1H), 4.16 (t, *J* = 7.7 Hz, 1H), 4.03 (dd, *J* = 7.9 Hz, *J* = 13.7 Hz, 1H), 3.70 (ddd, *J* = 5.4 Hz, *J* = 7.5 Hz, *J* = 13.1 Hz, 1H), 3.61 (ddd, *J* = 6.2 Hz, *J* = 7.9 Hz, *J* = 13.8 Hz, 1H), 3.29 (t, *J* = 6.5 Hz, 2H), 3.10 (br s, 2H), 2.85-2.64 (m, 4H + H_2O), 1.51-1.46 (m, 1H), 1.42-1.37 (2H), 1.33 (m, 1H), 1.10-1.04 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-*d*$_6$, 350 K, rotamers were observed): δ (ppm) 199.8 (C=O), 172.4 (C=O), 172.1 (C=O), 171.7 (C=O), 143.57 and 143.47 (2 x C_q, rotamers), 137.6 (C_q), 135.0 (C_q), 134.5 (CH), 134.0 (C_q), 129.6, 129.3, 129.2, 128.7, 127.8, 127.4, 126.9, 55.6 (CH), 54.2 (CH), 53.1 (CH), 50.1 (CH_2), 46.3 (CH_2), 39.5 (CH_2), 34.1 (CH_2), 32.1 (CH_2), 28.4 (CH_2), 27.5 (CH_2), 22.8 (CH_2). One CO and one CH_2 groups were not detected due to peak broadening or overlapping caused by the high temperature. Few peaks in the aromatic area could not be assigned, due to overlapping peaks. HRMS (ESI) m/z calcd for $C_{40}H_{45}N_4O_4$ $[M+H]^+$, 645.3435; found, 645.3431. HPLC purity, 100%, t_R = 18min, gradient A.

Biological characterization.

Membrane preparation for allosteric radioligand RAMX3 displacement and [^{35}S]GTPγS incorporation assays: The human embryonic kidney (HEK) cells stably expressing the human CXCR3 receptor were cultured in 150-mm cell culture plates in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% penicillin-streptomycin, 300 µg/mL Zeocin and incubated at 37 °C in a humid atmosphere with 5% CO_2. At 100% confluency cells were washed with PBS twice, treated with Tris EDTA buffer (10 mM Tris, 0.5 mM EDTA, 5 mM KCl, 140 mM NaCl, pH 7.4), and harvested using a cell scraper. Cells were pelleted at 1100×g for 8 min at 4 °C, resuspended in Tris-EDTA-$MgCl_2$ buffer (50mM Tris, 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM

KCl, 120 mM NaCl, pH 7.4) and followed by lysis with an Ultra-Turrax. After centrifugation at 50 000*g*, at 4°C for 18 min the membranes were resuspended in the binding buffer (50 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$ and subsequently homogenized with a glass-Teflon homogenizer (20 strokes). The homogenized membranes were shock-frozen in liquid nitrogen and stored at -80°C. The protein concentration was determined with the Lowry method with bovine serum albumin used as a standard.[49]

Allosteric radioligand displacement assay: Receptor binding studies were performed on membrane preparations of HEK cells expressing CXCR3. The tritium labeled RAMX3 (specific activity: 80.4 Ci/mmol) at the concentration of 1 nM was used for the assays. To determine unspecific binding 5µM of NBI-74330 was used. The assays were carried out in 96 well plates at a protein concentration of 30 µg/mL in a total volume of 200 µL. The incubation buffer contained 20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl and 0.1% BSA. (pH 7.4). After incubating for 1 h at 37°C, the binding was stopped by filtration through Whatman GF/B filters using a 96-channel cell harvester (Brandel, Unterföhring). The filters were rinsed five times with ice cold Tris-NaCl buffer. After drying for 3h at 60°C, filters were sealed with melt-on scintillator sheets Melti-Lex G/HS and the trapped radioactivity was measured in a microplate scintillation counter (Micro Beta Trilux scintillator). Three to five experiments per compound were performed with each concentration in triplicate. The *Kd* and *Bmax* values were determined in the homologous competition assay and have yielded following results: the *Kd* and *Bmax* values for the CXCR3 were 0.69 ± 0.13 nM and 5162 ± 367 fmol/mg.

[^{125}I]CXCL11 radioligand displacement assay: *Oxidative iodination.* The CXCL11 protein (purchased from PeproTech as the mature form of isoform 1) was labeled in-house with the ^{125}I isotope (purchased from Perkin-Elmer, in 10 µM NaOH, pH 8-11) using the Chloramine-T method.[50] CXCL11 was dissolved in 300 mM phosphate buffer, and 0.4 mCi

^{125}I was added to 0.6 nmol (10 µl) of the protein. 30 µl phosphate buffer supplemented with 30 mg/l Chloramine-T was added slowly over a 5 min period while stirring, and the reaction was quenched by adding 400 µl H_2O with 0.1% trifluoroacetic acid (TFA). The product was purified by reverse-phase HPLC, using a C18 column and a gradient from H_2O with 0.1% TFA (buffer A) to acetonitrile with 0.1% TFA (buffer B). After allowing the passage of 8 mL flow-through at 20 % buffer B, CXCL11 is eluted by increasing buffer B to 50 % over a flow-volume of 40 mL, after which any possible remains of protein are eluted by further increasing buffer B to 80 % across 10 mL flow-through. The flow-through is collected in 1 mL aliquots, and the radioligand is identified in those aliquots by gamma radiation intensity, and verified with a preliminary competitive binding assay setup.

Cell culture and transfection. Cos-7 cells expressing human CXCR3 (isoform 1) were used for binding assays. The cells were grown in DMEM 1885 (containing $NaHCO_3$) supplemented with 10% FBS, 0.029% L-Glutamine, 180 units/ml penicillin and 45 µg/ml streptomycin in tissue culture treated flasks (T75) to approximately 75% confluence. The calcium phosphate precipitation method[51] was used to transiently transfect the COS-7 cells before every experiment. 20 µg expression vector DNA (pcDNA3.1$^+$ encoding CXCR3 under control of the CMV promoter, or empty vector for negative controls) in 210 µl TE-buffer was mixed with 30 µl $CaCl_2$, and this was titrated into 240 µl HEPES buffered saline. The mixture was dripped directly unto the cells after a 45 min equilibration period, and the cells were incubated for 5 h in growth medium containing 30 mL/L chloroquine, after which it was exchanged for regular growth medium.

Competitive radioligand binding. Assay points were performed on transiently transfected COS-7 cells seeded one day earlier at 5.000 cells/well in poly-*D*-Lysine treated white 96-well plates. The cells were washed twice in 100 µl 50 mM HEPES buffer supplemented with 5 g/L BSA, then chilled to 4^{o}C for 15 min before addition of ligands, followed by addition of 10-15

pM radioligand aiming at 10% specific binding. Cells were incubated for 3 h at 4°C before being washed twice in 50 mM HEPES buffer with 5 g/L BSA and 29.22 g/L NaCl. 80 µl Gold Star (Liquid Scintillation Cocktail, Meridian, UK) was added per well before measuring remaining receptor-bound radioligand as scintillation on a Packard TopCount NXT.

ApoTox-Glo Triplex Assay: The ApoTox-Glo Triplex Assay (Promega) was performed according to the manufacturer's instructions. Briefly, 10 000 HEK293T cells stably expressing CXCR3 were seeded in a half-area white clear bottom 96-well plate and incubated overnight with compounds at the concentration of 10 µM in a final volume of 50 µL per well. After desired incubation time the viability/cytotoxicity reagent containing both GF-AFC and bis-AAF-R119 substrate were added to the well and incubated for additional 30 min. The fluorescence was measured at $400_{Ex}/505_{Em}$ (Viability) and $485_{Ex}/520_{Em}$ (Toxicity) with a microplate reader Clariostar (BMG labtech, Ortenberg, Germany). After the measurement the Caspase-Glo 3/7 reagent was added to all wells, incubated for 30 min and the luminescence measured with the microplate reader. The increase in the luminescence is indicative of caspase activation and thus a hallmark of apoptosis.

[^{35}S]GTPγS incorporation assay: The [^{35}S]GTPγS incorporation assay was performed on membrane preparations of stably transfected HEK293 cells that expressed the CXCR3 receptor. The assay was carried out in 96-well plates at the final volume of 200 µL. The incubation buffer contained 20 mM HEPES, 10 mM $MgCl_2$•$6H_20$,100 mM NaCl and 100 mg/L saponin (pH 7.4). Membranes (30 µg/mL of membrane protein), various concentrations of test compound and CXCL11, and 1 µM GDP were preincubated in the absence of [^{35}S]GTPγS for 30 min at 37 °C. After the addition of 0.10 nM [^{35}S]GTPγS membranes were incubated for additional 30 min at 37 °C. Incubation was terminated by filtration through Whatman GF/B filters soaked with ice cold PBS. The filter-bound radioactivity was

measured as described above. Three to four experiments per compound were performed with each concentration in triplicate.

β-arrestin 2 recruitment assay: The measurement of β-arrestin recruitment was performed utilizing the PathHunter assay purchased from DiscoveRx (DiscoveRx, Birmingham, U.K.) according to the manufacturer's protocol. HEK293 cells stably expressing β-gal enzyme acceptor were transiently transfected with the ProLink (PK1) tagged wildtype CXCR3 receptor using the TransIT-293 transfection reagent from Mirus (purchased from MoBiTec, Goettingen, Germany). The assay was carried out in 384-well plates containing 2000 cells in 20 µL. After incubation at 37 °C (5% CO_2, 95% relative humidity) overnight, various concentrations of test compounds or CXCL11 (prepared in PBS, pH 7.4, containing 0.20% BSA) were added and the incubation continued for 4 h. Detection mix was then added and incubated for further 60 min at room temperature. Chemiluminescence was determined with a microplate reader Clariostar (BMG labtech, Ortenberg, Germany).

Receptor internalization imaging: The day before transfection HEK293T cells were seeded in 6-well plates at a density of 200,000 cells/well. Transient transfection of the C-terminally eGFP-tagged CXCR3 cDNA was performed using TransIT293(MIRUS). Cells were transferred to poly-*L*-lysine coated glass coverslips 24 h after transfection. 2 d after transfection, cells were washed with phosphate buffered saline (PBS) and treated with the endogenous chemokine CXCL11 or compound **11** at 37°C in 5% CO2 for 60 min. Cells were fixed with 4% paraformaldehyde (RotiHistofix, Carl Roth) for 10min. After three washes with PBS, the glass coverslips were mounted on microscope slides using Dako Fluorescent Mounting Medium and images aquired using a Leica SP5 II confocal laser scanning microscope (Software LAS AF v2.7.3.9723.) equipped with Leica hybrid detectors. Excitation energy in all experiments was set to the same level to make all data set-ups

comparable in intensity. Post image processing (adjusting brightness and contrast) was performed for a better visualization.

Enzyme-linked immunosorbent assay (ELISA) based internalization assay. The day after transfection with FLAG-CXCR3, cells were detached with trypsin, resuspended into fresh culture medium and plated in poly-*D*-lysine-coated 48-well assay plates. 48h after transfection the medium was replaced by fresh DMEM without FBS that contained fixed concentration of the endogenous ligand CXCL11 (100 nM) or the positive allosteric modulators (10 μM) and incubated for 1h at 37°C. The cells were fixed with 4% formaldehyde solution and stained with monoclonal Anti-Flag antibody (Sigma-Aldrich Life Science, St. Louis, MO) and subsequently with Anti-Mouse IgG-Peroxidase Antibody produced in rabbit. *O*-phenylendiamine was used as a substrate for the enzyme coupled to the secondary antibody. The reaction was stopped with 1M H_2SO_4 and the resulting color was detected at 492 nm using a microplate reader Clariostar (BMG labtech, Ortenberg, Germany).

Transwell migration assay. HEK-293 cells were maintained in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin. For DNA transfection, 5 μg plasmid DNA (CXCR3) was mixed with TE buffer to give a final of 105 μl solution and 15 μl $CaCl_2$ (2M) was added. The DNA mixture was then dripped into 120 μl 2x HBSS solution, and the mixture was allowed to form calcium-phosphate precipitates for 45 minutes. The DNA/calcium-phosphate precipitate was dripped onto HEK293 cells (50% confluent in T25 flask), where the medium had been renewed and reduced to 1.5 ml 30 minutes prior to transfection. The cells were split and used for assays 48 h after transfection.

Migration of serum-starved HEK293 cell transiently transfected with CXCR3 was assessed using Transwell™ membranes (Costar; 8-μm pore). Filters were coated with 10 μg/mL fibronectin dissolved in PBS for 30 minutes at room temperature). The fibronectin solution was removed and the filters allowed to air-dry. The filters were placed in a 24-well dish that

contained low serum (0.2%) DMEM supplemented with agonists or control buffer. HEK293 cells suspended in 0.2% serum DMEM containing test compounds were added to the upper chamber (1×10^5 cells/well). Cells were allowed to migrate for 5 h at 37 °C. Nonmigrated cells were removed from the top filter surface with a cotton swab. Migrated cells, attached to the bottom surface, were fixed in 3.7% formaldehyde (in PBS) and dyed with crystal violet. Transmigrated cells were counted in 5 predefined areas on the membrane using 20X objective.

Data analysis. The resulting competition curves of the allosteric radioligand displacement and activity assays were analyzed by nonlinear regression using the algorithms in PRISM 5.0 (GraphPad Software, San Diego, CA). Competition curves were fitted to the sigmoid curve by non-linear regression analysis in which the $\log EC_{50}$ value and the Hill coefficient were free parameters.

KEYWORDS: chemokine receptor, CXCR3, tetrahydroisoquinoline carboxamides, allosteric agonists, biased signaling

ASSOCIATED CONTENT

Supporting Information. Supplementary figures: toxicity data, characterization of CXCR3-PK1 and CXCR3-PK2, [^{125}I] CXCL11 and allosteric radioligand RAMX3 displacement assays data; synthetic procedures for compounds **34** (FAUC1095), **46** and **47**, purity data and spectral characterization of final compounds and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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ABBREVIATIONS

ß-arr. 2, ß-arrestin 2; Boc, tert-Butyloxycarbonyl; CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; CD4, immune cells (Cluster of differentiation 4); CXCR3, CXC chemokine receptor 3; CXCL11, CXC chemokine ligand 11; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMF, dimethylformamide; EDC hydrochloride, N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; HEK293, human embryonic kidney 293 cells; Hex, hexane; TOF, time-of-flight mass spectrometry.

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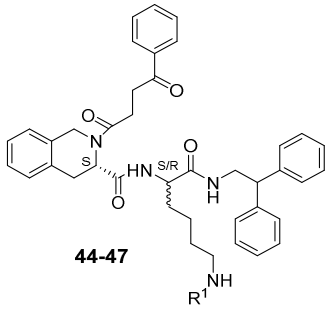
Tables

Table 1. The ability of the 46 based positive allosteric modulators to activate CXCR3 as measured in the [^{35}S]GTPγS incorporation assay.[a]

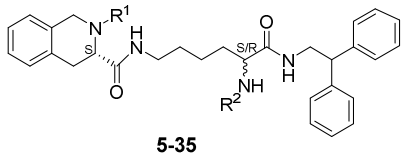


44-47

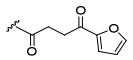
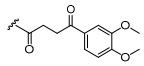
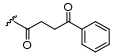
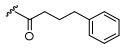
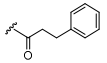
Cpd	Config.	R^1	CXCR3	
			pEC_{50} ± SEM[b]	Efficacy (%) ± SEM[c]
CXCL11	-	-	8.88±0.13	100
44	*S,S*	Boc	5.88±0.10	47±3
45	*S,R*	Boc	5.97±0.34	33±7
46	*S,S*	H	6.20±0.06	109±3
47	*S,R*	H	5.94±0.09	85±5

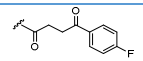
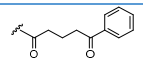
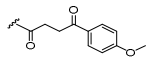
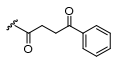
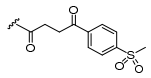
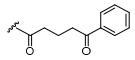
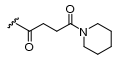
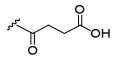
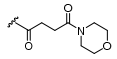
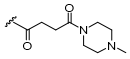
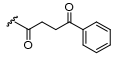
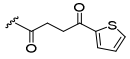
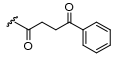
[a] The ability of positive allosteric modulators to activate CXCR3 was determined by the [^{35}S]GTPγS incorporation assay with the membrane preparations of HEK293T stably expressing CXCR3. [b] The values reflect the mean pEC_{50}±SEM of three experiments performed in triplicate. [c] The efficacy represents the % of CXCL11 mediated effect.

Table 2. The ability of the compound 5 based positive allosteric modulators to activate CXCR3 as measured in the [^{35}S]GTPγS incorporation assay.[a]



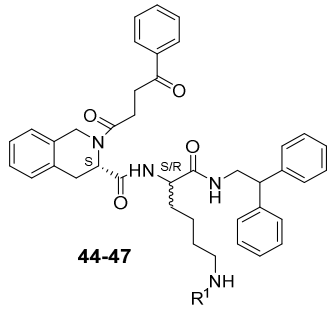
5-35

Cpd	R^1	R^2	Config.	CXCR3		Cpd	R^1	R^2	Config.	CXCR3	
				pEC_{50} ± SEM[b]	Efficacy (%) ± SEM[c]					*pEC_{50}* ± SEM[b]	Efficacy (%) ± SEM[c]
CXCL11				8.88±0.13	100	**20**		Boc	S,S	No effect	No effect
5	H	Boc	*S,S*	5.66±0.34	34±8	**21**		Boc	S,S	7.30±0.17	18±1
11		Boc	*S,S*	No effect	No effect	**22**		Boc	S,S	5.07±0.50	29±15
12		Boc	*S,S*	No effect	No effect	**23**		Boc	S,S	5.07±0.50	30±17

13		Boc	*S,S*	6.29±0.31	20±3	**24**		Boc	S,S	No effect	No efffect
14		Boc	*S,S*	5.22±0.31	50±19	**25**		H	S,S	No effect	No effect
15		Boc	*S,S*	5.91±0.20	25±3	**26**		H	S,S	No effect	No effect
16		Boc	*S,S*	No effect	No effect	**27**		Boc	S,S	No effect	No effect
17		Boc	*S,S*	No effect	No effect	**28**	H	H	S,S	5.65±0.23	40±7
18		Boc	*S,S*	No effect	No effect	**34**		Boc	S,R	No effect	No effect
19		Boc	*S,S*	No effect	No effect	**35**		H	S,R	8.01±0.31	25±4

[a] The ability of positive allosteric modulators to activate CXCR3 was determined by the [^{35}S]GTPγS incorporation assay with membrane preparations of HEK293T stably expressing CXCR3. [b] The values reflect the mean *pEC50*±SEM of three experiments performed in triplicate. [c] The efficacy represents the % of CXCL11 mediated effect.

Table 3. Ability of the compound 46 based positive allosteric modulators to activate the CXCR3 as measured in the β-arrestin 2 recruitment assay (PathHunter, DiscoveRx).[a]

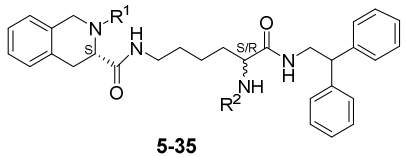


44-47

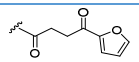
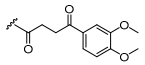
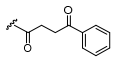
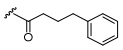
Cpd	Config.	R^1	CXCR3-PK1	CXCR3-PK2
			pEC_{50} ± SEM[b] (%)[c]	pEC_{50} ± SEM (%)[c]
CXCL11			7.76±0.06 (100)	7.75±0.17 (100)
44	*S,S*	Boc	>5.0 (70%@10μM)	nd[d]
45	*S,R*	Boc	6.63±0.17 (36±2)	7.23±0.69 (30±9)
46	*S,S*	H	5.89±0.07 (139±5)	nd[d]
47	*S,R*	H	5.96±0.08 (108±5)	6.12±0.11 (198±12)

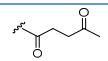
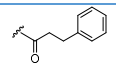
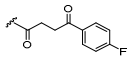
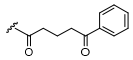
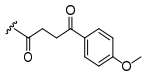
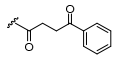
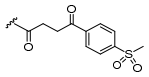
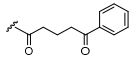
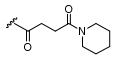
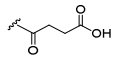
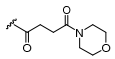
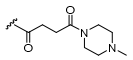
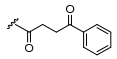
[a]The ability of the positive allosteric modulators to recruit β-arrestin 2 to CXCR3 was determined with HEK293-T cells expressing all of the components of PathHunter (DiscoveRx) and CXCR3-PK1 or CXCR3-PK2. [b] The values reflect the mean pEC_{50} ± SEM of two to three experiments performed in triplicate. [c] The efficacy represents the % of CXCL11 mediated effect. [d] Not determined.

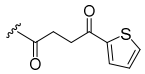
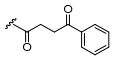
Table 4. Ability of the compound 5 based positive allosteric modulators to activate the CXCR3 as measured in the β-arrestin 2 recruitment assay (PathHunter, DiscoveRx).[a]



5-35

Cpd	R^1	R^2	Config.	CXCR3-PK1 pEC_{50} ± SEM[b] (%)[c]	CXCR3-PK2 pEC_{50} ± SEM[b] (%)[c]	Cpd	R^1	R^2	Config.	CXCR3-PK1 pEC_{50} ± SEM[b] (%)[c]	CXCR3-PK2 pEC_{50} ± SEM (%)[c]
CXCL11				7.76±0.06 (100)	7.75±0.17 (100)	**20**		Boc	*S,S*	No effect	nd[d]
5	H	Boc	*S,S*	6.50±0.17 (31±2)	6.71±0.33 (79±12)	**21**		Boc	*S,S*	No effect	nd[d]
11		Boc	*S,S*	6.64±0.38 (30±5)	6.58±0.30 (66±8)	**22**		Boc	*S,S*	No effect	nd[d]

12		Boc	*S,S*	6.78±0.17 (34±2)	6.64±0.33 (63±9)	**23**		Boc	*S,S*	7.21±0.17 (19±1)	6.71±0.30 (29±4)
13		Boc	*S,S*	No effect	nd[d]	**24**		Boc	*S,S*	6.84±1.7 (20±2)	6.78±0.35 (56±9)
14		Boc	*S,S*	No effect	nd[d]	**25**		H	*S,S*	6.80±0.21 (17±1)	6.51±0.34 (27±3)
15		Boc	*S,S*	No effect	nd[d]	**26**		H	*S,S*	6.73±0.22 (20±2)	6.90±0.21 (91±9)
16		Boc	*S,S*	No effect	nd[d]	**27**		Boc	*S,S*	No effect	No effect
17		Boc	*S,S*	No effect	nd[d]	**28**	H	H	*S,S*	6.80±0.45 (14±3)	6.55±0.26 (85±10)
18		Boc	*S,S*	No effect	nd[d]	**34**		Boc	*S,R*	6.95±0.50	6.86±0.19

										(16±3)	(88±8)
19		Boc	*S,S*	No effect	nd[d]	**35**		H	*S,R*	7.16±0.33 (28±4)	6.52±0.43 (79±16)

[a]The ability of the positive allosteric modulators to recruit β-arrestin 2 to CXCR3 was determined with HEK293-T cells expressing all of the components of PathHunter (DiscoveRx) and CXCR3-PK1 or CXCR3-PK2. [b] The values reflect the mean pEC_{50} ± SEM of two to three experiments performed in triplicate. [c] The efficacy represents the % of CXCL11 mediated effect. [d] Not determined.

Scheme Legends

Scheme 1. Synthesis of Precursor 5[a]

[a]Reagents and Conditions: (i) diphenyl ethylamine, EDC hydrochloride, DCM, 30 min, 0 °C, 16 h, rt, 80%; (ii) H_2, 10% Pd/C, MeOH, 6 h, rt, 99%; (iii) benzyl chloroformate, NaOH, dioxane, 30 min, 0 °C, 16 h, rt, 99%; (iv) EDC hydrochloride, DCM, 30 min: 0 °C 16 h: rt, 56%; (v) H_2, 10% Pd/C, MeOH, 6 h, rt, 98%.

Scheme 2. Preparation of heterocyclic acids[a]

[a]Reagents and Conditions: (i) succinic anhydride, DCM, 2 h, rt, 86-95%; (ii) succinic anhydride, $AlCl_3$, 90 min, 0 °C, 4-5 h, rt, 57-63%.

Scheme 3. Synthesis of Final Compounds using Precursor 5[a]

[a]Reagents and Conditions: (i) acid, EDC hydrochloride, DIPEA, DCM, 30 min, 0 °C, 15-24 h, rt, 55-95%; (ii) succinic anhydride, DCM, 18 h, rt, 81%; (iii) trifluoroacetic acid, DCM, 3 h, rt, 32-75%;

Figure Legends

Figure 1. Previously reported CXCR3 agonists: tetrahydroisoquinoline **46**[17,19] and **48**[17], pyrazinodiazepine (**49**)[17], and biaryl type ligands (**50, 51**)[52].

Figure 2. Investigation of structural features for biased allosteric agonists based on the scaffold of **11**.

Figure 3. Strongly biased allosteric agonists of CXCR3.

Figure 4. Internalization of CXCR3 upon treatment with **11**. A) In untreated cells, CXCR3 is mostly located at the cell surface. Stimulation with 100 nM endogenous ligand CXCL11 resulted in the internalization of CXCR3. Treatment with **11** at concentrations 10 μM and 1 μM led to the internalization of a significant portion of the receptors, but not the treatment with **14**. The scale bars in panel represent 20μm. B) Quantification of receptor internalization upon the treatment with the endogenous agonist CXCL11, **11** and **14**, where only **11** and CXCL11 led to receptor internalization. Statistical analysis was performed with Student t test (**p <0.01).

Figure 5. 11 and **14** do not influence the binding of CXCL11 to CXCR3. A) Competition binding assay with **11** and **14** using 10-15 pM of [^{125}I]CXCL11 as radioligand. B) Competition binding assay with [^{125}I]CXCL11 (10-15 pM) as the radioligand in the presence of 1000 nM of **11** and **14**. C) **11** causes the CXCR3 receptor desensitation. The CXCR3 expressing HEK293T cells were incubated with given concentrations of **11** and various concentrations of CXCL11 and the β-arrestin 2 recruitment was measured. Curves represent pooled and normalized data of three experiments performed in triplicate.

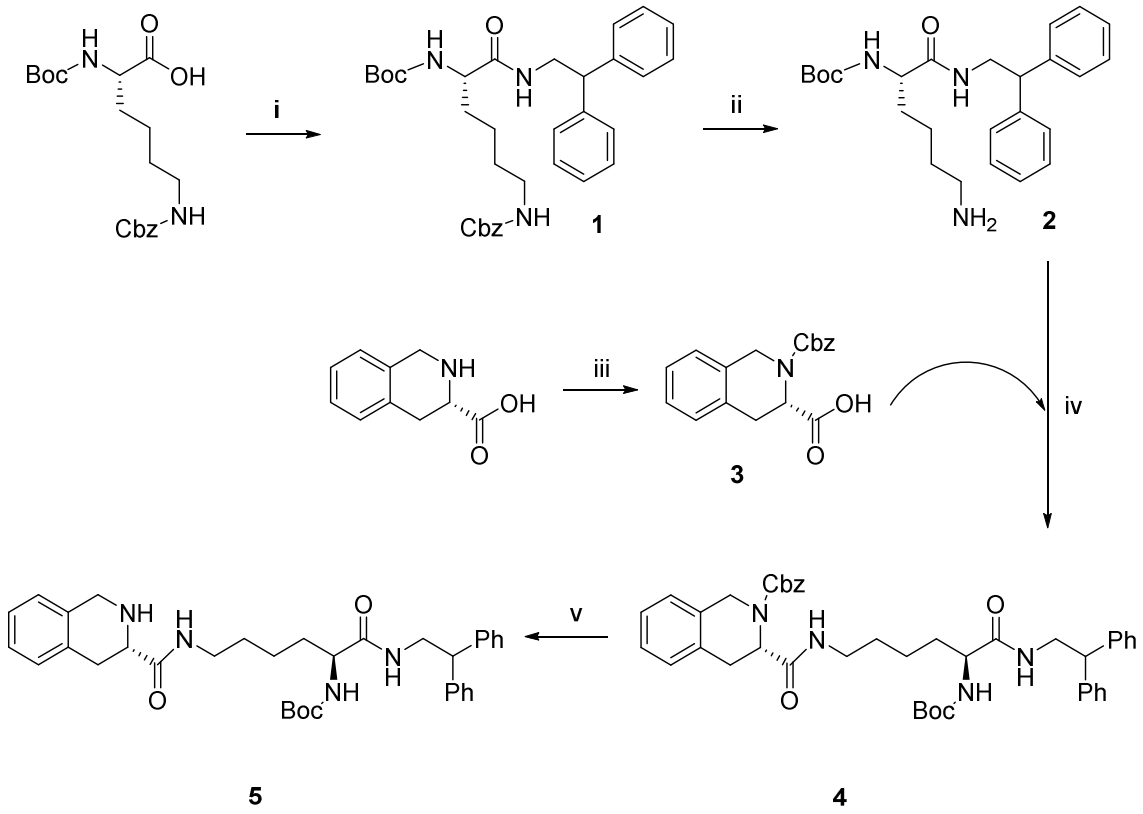
Figure 6. 11 and **14** induce directed cell migration. A) Using a standard transwell assay we quantified migration of HEK293 cells transiently transfected to express CXCR3 against CXCL11 as positive control as well as **11** and **14**. Migration against CXCL11 follows a classical dose response with peak migration at 1 nM CXCL11. **11**

and **14** both induced migration on their own with peak migration at 100 nM compound concentrations in the sink reservoir. B) **11** and **14** both seem to positively influence CXCL11 induced migration when present in both source and sink during experimentation, but only **14** reached significance in the current setup; BC: buffer control. Statistical analysis was performed with Student t test (*p<0.05, **p <0.01, ***p<0.001).

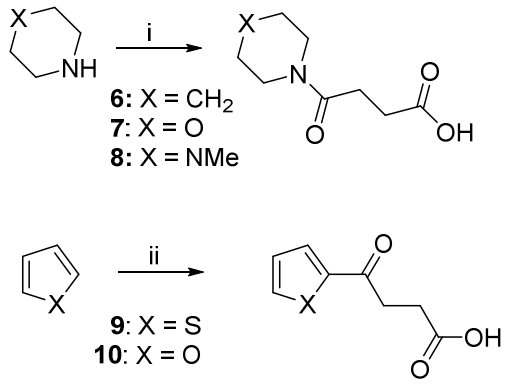
Figure 7. Tetrahydroisoquinoline scaffold based pharmacophore model depicting crucial structural elements that dictate biased agonism on allosteric agonists of CXCR3.

Schemes

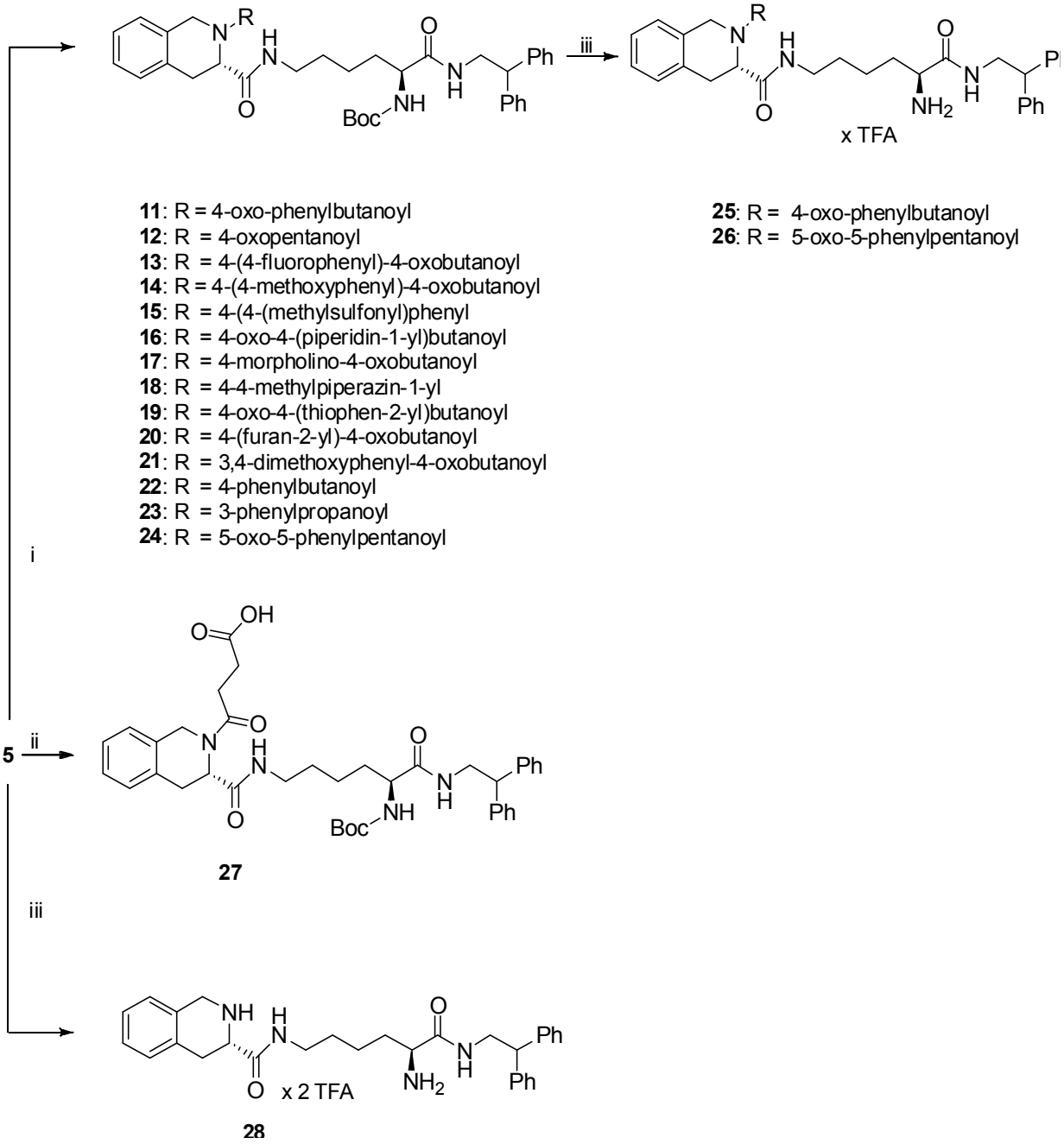
Scheme 1. Synthesis of Precursor 5[a]



Scheme 2. Preparation of the Heterocyclic acids[a]

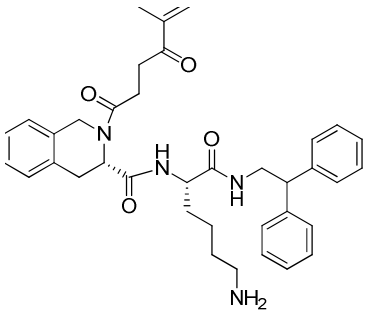


Scheme 3. Synthesis of Final Compounds using Precursor 5[a]

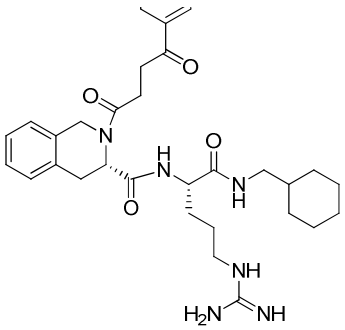


Figures

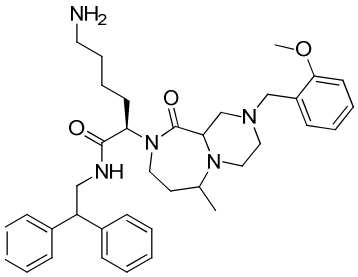
Figure 1



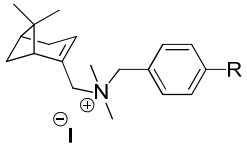
46 (VUF10661)



48 (PS372424)



pyrazinodiazepine derivative **49**



biaryl derivative **50**: R = 2-Br-Ph
biaryl derivative **51**: R = 2-I-Ph

Figure 2

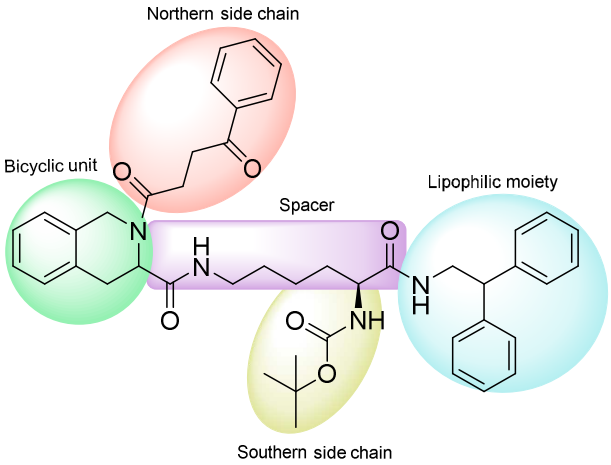

Northern side chain
Bicyclic unit
Spacer
Lipophilic moiety
Southern side chain

Figure 3

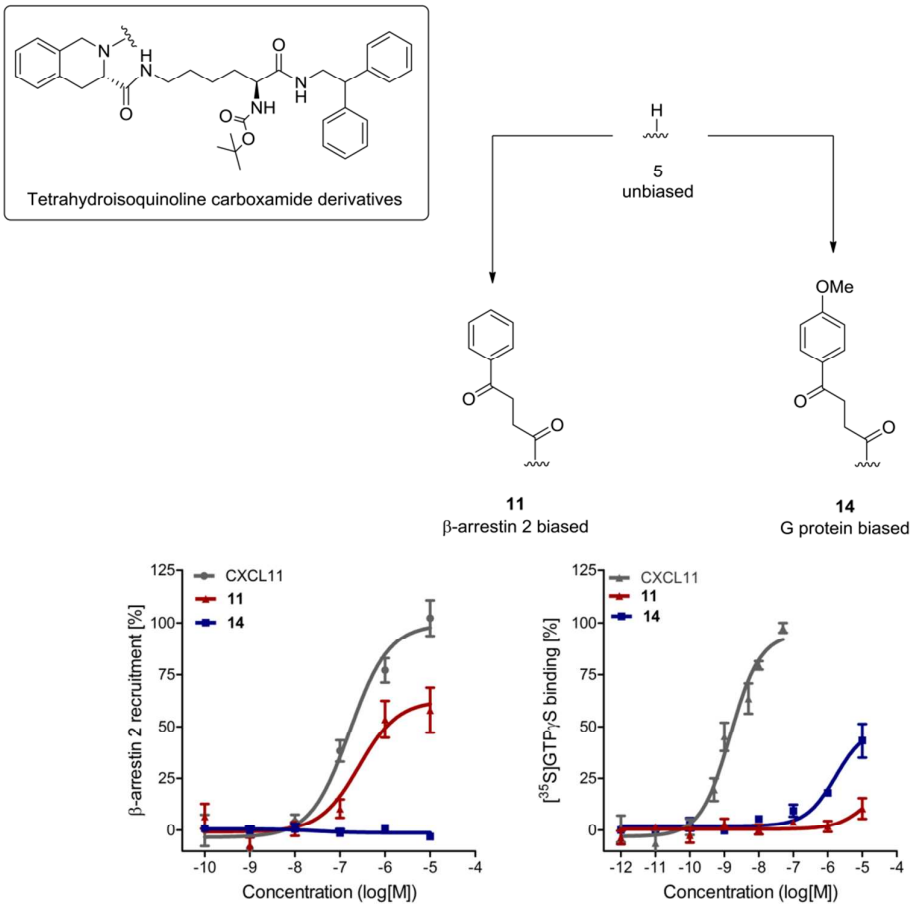

Tetrahydroisoquinoline carboxamide derivatives
H
5
unbiased
OMe
11
β-arrestin 2 biased
14
G protein biased
CXCL11
11
14
β-arrestin 2 recruitment [%]
Concentration (log[M])
[35S]GTPγS binding [%]
Concentration (log[M])

Figure 4

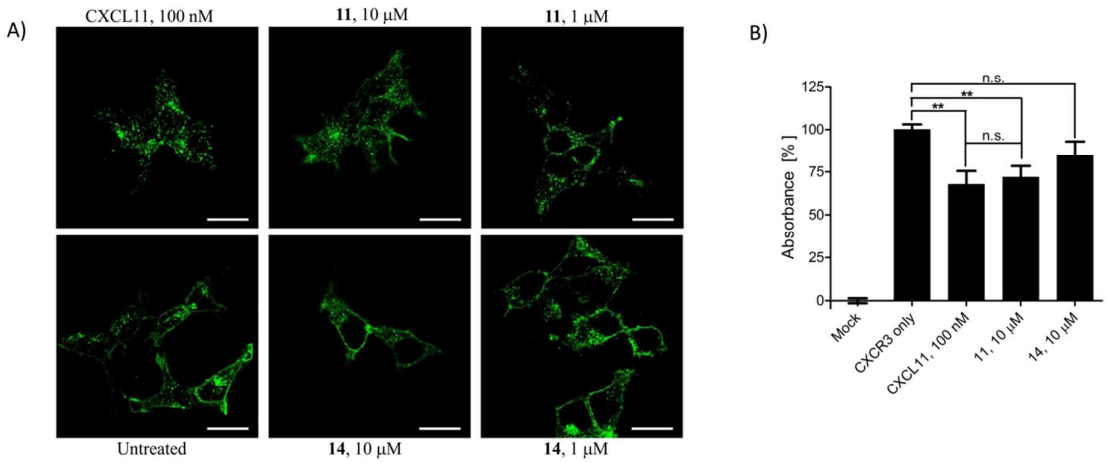


Figure 5

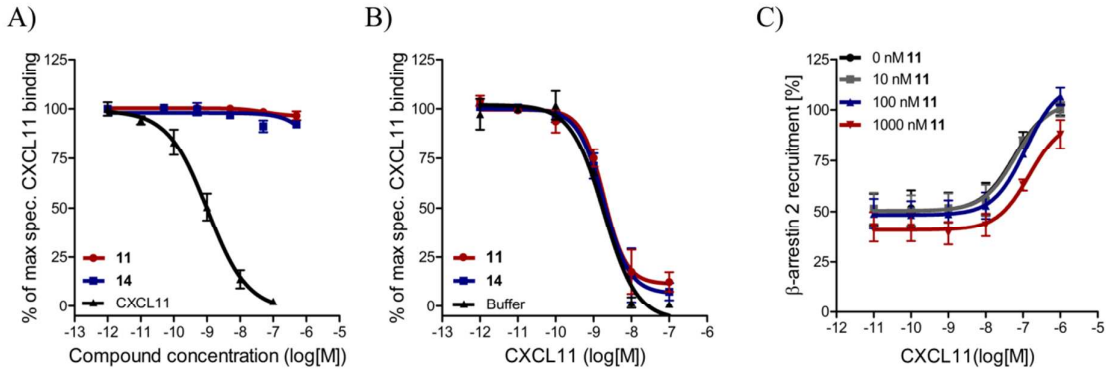


Figure 6

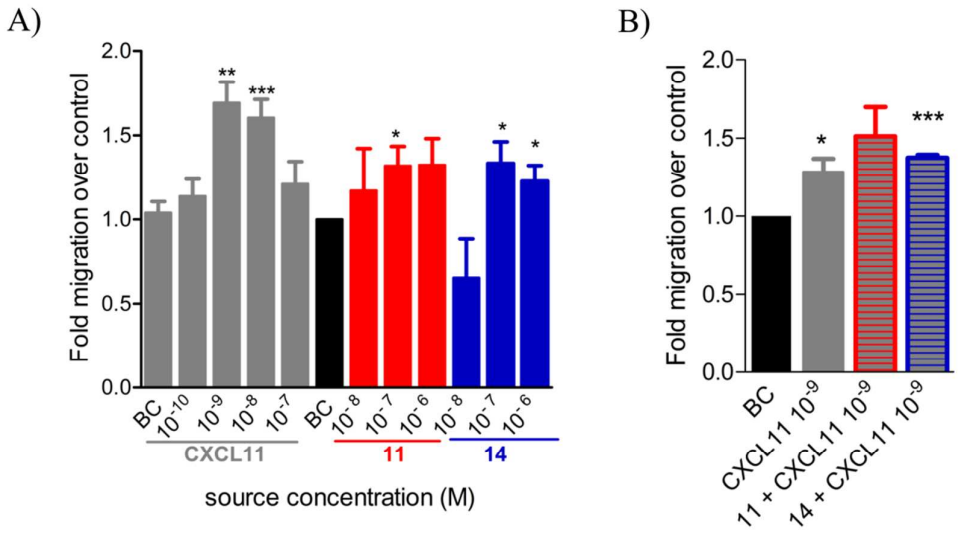
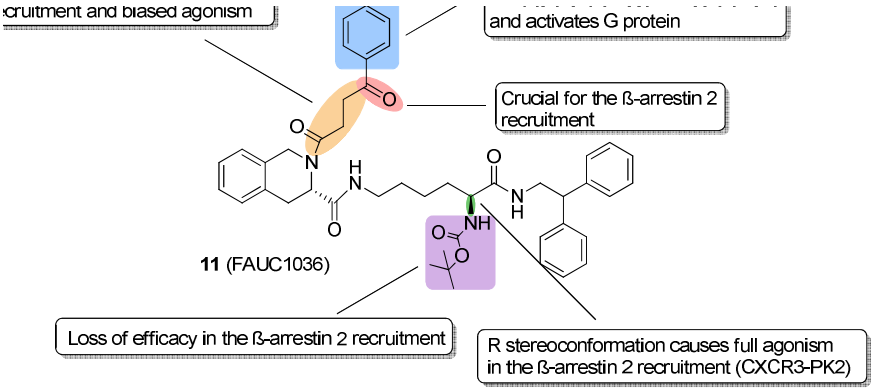
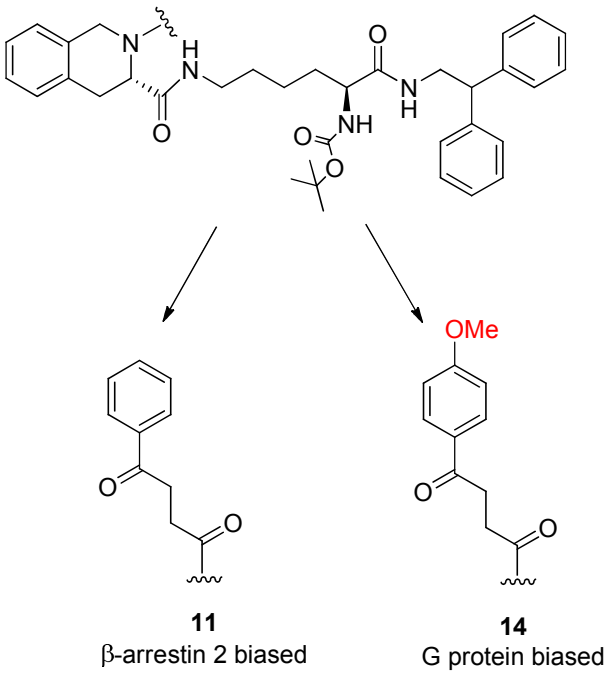

A)
Fold migration over control
2.0
1.5
1.0
0.5
0.0
**
***
*
*
*
BC
10-10
10-9
10-8
10-7
BC
10-8
10-7
10-6
10-8
10-7
10-6
CXCL11
11
14
source concentration (M)
B)
Fold migration over control
2.0
1.5
1.0
0.5
0.0
*
***
BC
CXCL11 10-9
11 + CXCL11 10-9
14 + CXCL11 10-9

Figure 7


ɔcruitment and biased agonism
and activates G protein
Crucial for the ß-arrestin 2 recruitment
11 (FAUC1036)
Loss of efficacy in the ß-arrestin 2 recruitment
R stereoconformation causes full agonism in the ß-arrestin 2 recruitment (CXCR3-PK2)

Insert Table of Contents Graphic and Synopsis Here



11
β-arrestin 2 biased

14
G protein biased