**Synthesis of a novel tripeptidomimetic scaffold and biological evaluation for CXC chemokine receptor 4 (CXCR4) antagonism**

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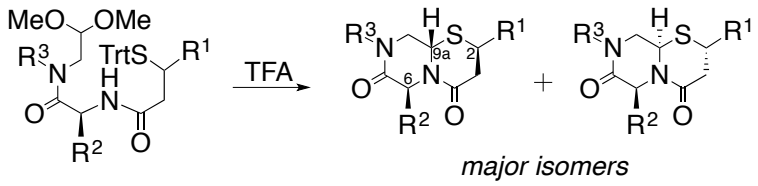
**Abstract**

We here report the preparation of a new 2,6,8-trisubstituted bicyclic tripeptidomimetic scaffold through TFA-mediated cyclization of a linear precursor containing three side chains. The introduction of a triphenylmethyl-protected thiol into carboxylic acid containing building blocks through sulfa Michael additions onto α,β-unsaturated hexafluoroisopropyl esters is described. The stereoselectivity of the bicycle formation was found to be somewhat lower than that previously reported for analogous 3,6,8-trisubstituted scaffolds. Moreover, the configuration of the linear precursor directs the stereochemical outcome of the cyclization differently when the $R^1$ side chain is positioned on C2 in the bicycles (present work) instead of C3 (previous work). Tripeptidomimetic compounds based on the new scaffold were synthesized and evaluated for antagonistic potency toward CXCR4, and one compound (**45a**) displayed similar activity to earlier reported 3,6,8-tripeptidomimetic bicycles.

**Graphical abstract:**



## 1. Introduction

Endogenous and exogenous peptides display a multitude of important activities related to human disease states, and both the processing of peptide substrates and the binding of signalling peptides have been targeted for therapeutic purposes. Thus, proteolytic enzymes (proteases) as well as peptide-binding (peptidergic) receptors are targets for drugs. Compounds that interfere with either processing or signalling of peptides are commonly referred to as peptidomimetics, and have been classified as either peptide backbone mimetics (type I), functional mimetics (type II) or topographical mimetics (type III).[1] Peptidomimetic structures have found widespread use as drugs, and in a drug discovery perspective, tripeptidomimetics have been identified as particularly interesting.[2]

We have previously reported the synthesis of a novel type of tripeptidomimetics based on a 3,6,8-trisubstituted 4,7-dioxo-1-thia-5,8-diaza-bicyclo[4.4.0]decane scaffold (Figure 1A).[3] These compounds were Arg[1]-Arg[2]-2-Nal[3] (2-napthylalanine, 2-Nal) mimetics and were designed as antagonists for the CXC chemokine receptor 4 (CXCR4), which is a 7TM receptor involved in entry of T-tropic HIV strains into $CD4^+$ T-cells,[4,5] and also has a pathological role in cancer and rheumatoid arthritis.[6] The prototype compounds **1a** and **1b** (Figure 1A) were shown to have $EC_{50}$ values of 64 μM and 80 μM, respectively, in a cell-based assay for wild-type CXCR4.[3] We recently reported an array of close analogues aimed at investigating the influence of side chain length (m = 2-4, n = 3-4, o = 1-2) on antagonistic potency, where **2a,b** (Figure 1A; isolated as a mixture of diastereoisomers) showed an $EC_{50}$ of 61 μM.[7]

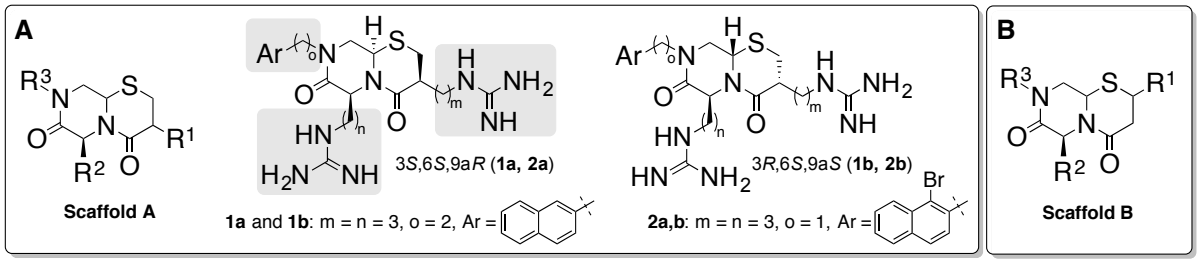



**Figure 1.** A) Bicyclic scaffold A and tripeptidomimetic CXCR4 antagonists based on scaffold A[3,7]; B) Bicyclic scaffold B.

Our tripeptidomimetic compounds are inspired by a series of highly potent cyclopentapeptide CXCR4 antagonists originally reported by Fujii et al.[8] The most potent cyclopentapeptides were based on the amino acid sequence L/D-Arg[1]-Arg[2]-2-Nal[3]-Gly[4]-D-Tyr[5], where the Arg[1]-position was shown to be relatively insensitive to configurational change, as both L- and D-Arg[1] were tolerated.[8] Later, Demmer et al. showed

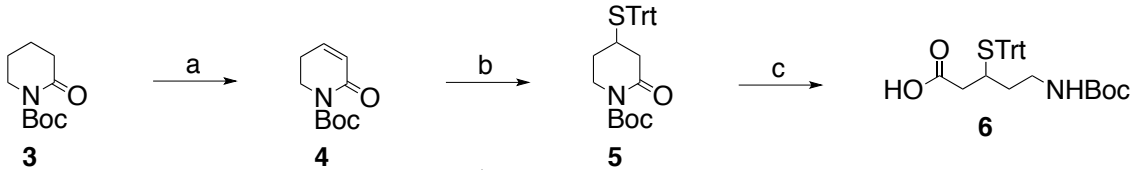
that re-positioning of the $Arg^1$ side chain to give *N*-alkylated D-$Ala^1$ cyclopentapeptide analogues resulted in high antagonistic potency.[9] For our approach, we envisioned that the 2,6,8-trisubstituted scaffold B (Figure 1B) would be suited to present the side chains of the $Arg^1$-$Arg^2$-$Nal^3$ tripeptide fragment in a similar manner as for the cyclopentapeptides. In this scaffold, the $C_2$-$C_3$ bond would correspond to the Cα-Cβ vector of the $Arg^1$ side chain, resulting in an additional conformational constraint. Our synthetic endeavours toward this novel scaffold are reported herein.

## 2. Results and Discussion

### 2.1. Synthesis

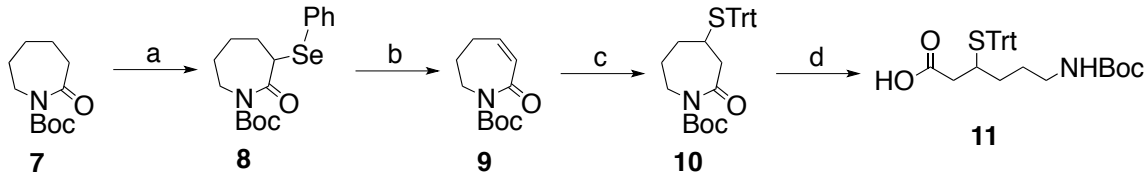
In our previous syntheses of tripeptidomimetic compounds, the building blocks bearing the $R^1$ side chain were prepared without any attempts at controlling the configuration of the chiral centre.[7,3] Consequently, two diastereoisomers (**a** and **b**, Figure 1A) of the scaffold A bicycles were formed in the cyclization. The sulfa Michael-type addition of the deprotected thiol onto the acyliminium ion intermediate was highly stereoselective, as initially investigated by Grimes et al.,[10] and resulted in two diastereomeric bicycles where H6 and H9a always displayed a *cis* relationship.[7,3]

In our earlier syntheses of building blocks for scaffold A with different chain length for the $R^1$ side chain, the sulfur atom was introduced through a Michael addition between triphenylmethanethiol and *N*-Boc protected α-methylenyl lactams of varying ring size.[3,7] Subsequent hydrolysis of the *N*-Boc protected lactams[11] gave the required *N*-Boc protected ω-amino carboxylic acids. For scaffold B, we envisioned that a Michael acceptor with an endocyclic double bond would give access to an array of building blocks with varying methylene spacer length for the $R^1$ side chain. Toward this end, we started out by converting *N*-Boc lactam **3** into α,β-unsaturated lactam **4** through reaction of the corresponding enolate with *N*-*tert*-butylbenzenesulfinimidoyl chloride at −78 °C (Scheme 1).[12] The α,β-unsaturated lactam **4** was difficult to separate from the starting material and was isolated as a ~10:1 mixture with **3**; however, in the subsequent addition step, the desired Michael adduct **5** could be isolated in pure form. Finally, hydrolysis of **5** gave carboxylic acid **6** in high yield.



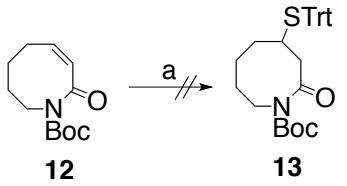

**Scheme 1.** *Reagents and conditions:* a) i) LDA, THF, -78 °C ii) PhS(Cl)=N-*t*-Bu, THF, -78 °C (90%, **4**/**3** ~10:1); b) $Ph_3CSH$, $Et_3N$, $CH_2Cl_2$ (68%); c) 1 M aq. LiOH, THF (82%).

For elongation of the $R^1$ side chain, *N*-Boc protected lactam **7** (Scheme 2) was treated with LiHMDS in dry THF to form the corresponding enolate, which was reacted with phenylselenyl chloride to give phenylselenide **8**.[13] Oxidation with hydrogen peroxide to the selenoxide gave in turn the α,β-unsaturated lactam **9**. However, this material proved to be difficult to purify, and the crude product was therefore used directly in the Michael addition.[14] Triphenylmethanethiol was used as the Michael donor in the presence of triethylamine as base and dry $CH_2Cl_2$ as solvent, but this reaction was unsuccessful. By changing the solvent to anhydrous DMF, the Michael product **10** was obtained in 66% yield over two steps. Finally, hydrolysis gave carboxylic acid **11**.



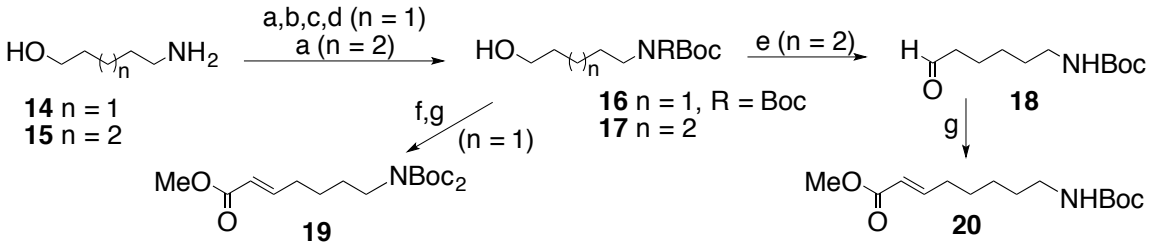

**Scheme 2.** *Reagents and conditions:* a) i) LiHMDS, THF, -78 °C, ii) PhSeCl, THF, -78 °C (86%); b) $H_2O_2$, THF, 0 °C; c) $Ph_3CSH$, $Et_3N$, DMF (66% over two steps); d) 1 M aq. LiOH, THF (71%).

While the Michael addition worked well both for the six- and seven-membered lactams, reaction with **12** (Scheme 3) did however not proceed according to plan (data not shown). Although the *m*/*z* for the Michael product **13** was observed for the crude product, all attempts at isolating the product by flash column chromatography proved to be unsuccessful. One reason for the poor outcome might be lower ring-strain for the eight-membered α,β-unsaturated lactam **12** leading to lower reactivity than the six- and seven-membered analogues. Since we were planning to prepare bicyclic analogues with an $R^1$ side chain containing five methylene groups, which would require Michael addition onto an α,β-unsaturated 9-membered lactam, we decided to abandon the lactam strategy.



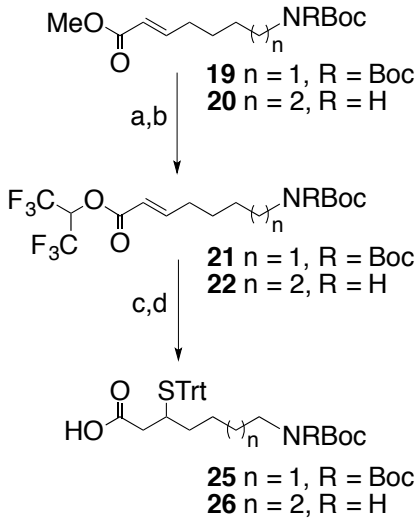

**Scheme 3.** *Reagents and conditions:* a) $Ph_3CSH$, $Et_3N$, DMF.

In order to access derivatives with a longer methylene chain for the $R^1$ group, we decided to employ α,β-unsaturated methyl esters **19** and **20** as Michael acceptors (Scheme 4), which were prepared starting from amino alcohols **14** and **15** through *N*-protection[15] followed by oxidation to aldehydes and then Wittig reaction with methyl (triphenylphosphoranylidene)acetate (Scheme 4).



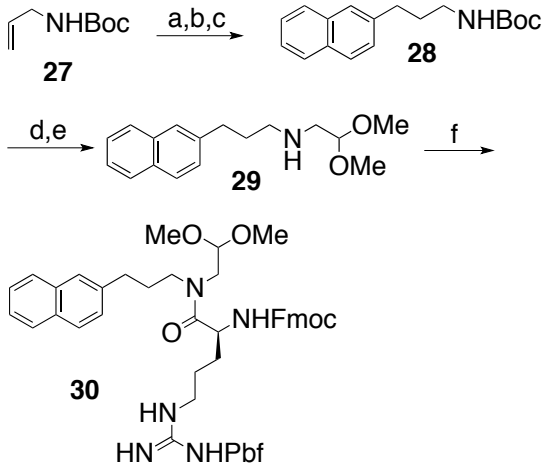

**Scheme 4.** *Reagents and conditions:* a) $Boc_2O$, 1,4-dioxane, 0 °C to rt; b) TBDMSCl, imidazole, $CH_2Cl_2$, 0 °C; c) i) *n*-BuLi, THF, 0 °C, ii) $Boc_2O$, THF, 0 °C to rt; d) TBAF, THF (**16**: 52% over 4 steps); e) TEMPO, *n*-$Bu_4NCl$, NCS, $CH_2Cl_2$ (**18**: 37% over 2 steps); f) Dess-Martin periodinane, $CH_2Cl_2$, 0 °C; g) $MeOC(O)CHP(Ph)_3$, THF, 60 °C (**19**: 81% over 2 steps; **20**: 57%).

Michael addition with triphenylmethanethiol onto α,β-unsaturated methyl esters (such as **19** and **20**) proved to be very unreliable. Complete conversion of the starting material could never be achieved, albeit a variety of different conditions were tested.[16] In addition, triphenylmethanethiol decomposed during the reactions to give products with similar $R_f$ values as the desired Michael addition products, which hampered purification by flash column chromatography. As the methyl esters **19** and **20** proved to be too unreactive, we decided to increase the electrophilicity by conversion into hexafluoroisopropyl esters **21** and **22** (Scheme 5) through DCC mediated esterification.[17] The Michael addition now gave protected thiols, which were hydrolysed to carboxylic acids **25** and **26**.




**Scheme 5.** *Reagents and conditions:* a) 1) 1 M aq. LiOH, THF; b) 1,1,1,3,3,3-hexafluoropropan-2-ol, DMAP, DIC, $CH_2Cl_2$ (**21**: 62%; **22**: 61%, both over 2 steps); c) $Ph_3CSH$, $Et_3N$, $CH_2Cl_2$ (**23**: 52%; **24**: 91%); d) 1 M aq. LiOH, THF (**25**: 33%; **26**: 56%).

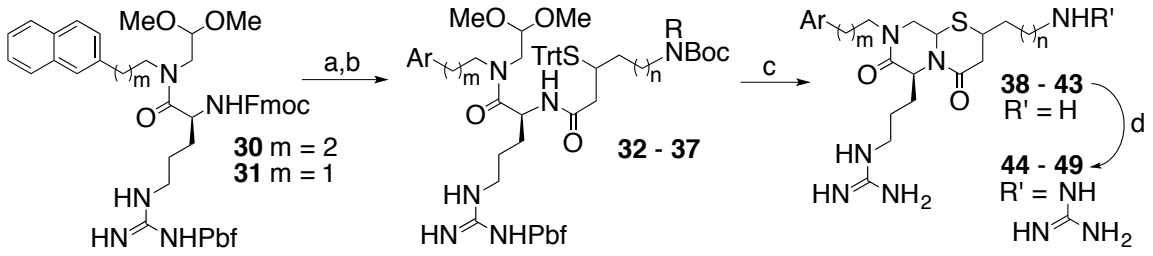
In order to elongate the $R^3$-side chain compared to our previously reported antagonists based on scaffold A,[3] secondary amine **29** was prepared from *N*-Boc protected allylamine **28** through hydroboration followed by Suzuki-Miyaura coupling and then Boc-deprotection followed by alkylation (Scheme 6).[18]




**Scheme 6.** *Reagents and conditions:* a) 9-BBN, THF; b) 2-Bromonapthalene, $Pd(dppf)_2Cl_2$•$CH_2Cl_2$, 1 M NaOH; c) NaOH, $H_2O_2$, $H_2O$, THF, 0 °C (70% over 3 steps); d) TFA, $CH_2Cl_2$, 0 °C; e) $BrCH_2CH(OMe)_2$, $K_2CO_3$, DMF, 80 °C (31% over 2 steps); f) Fmoc-Arg(Pbf)-OH, HATU, DIPEA, $CH_2Cl_2$ (79%).

Next, the $R^1$ side chain bearing carboxylic acids were coupled with the Fmoc-deprotected $R^2$-$R^3$ fragments **30** and **31**[3] (Scheme 7). The resulting linear precursors **32**-**37** (inseparable mixture of diastereoisomers) were submitted to TFA-mediated deprotection and cyclization to give bicyclic compounds **38**-**43**, which were

guanidinylated to give the final products **44-49** as mixtures of diastereoisomers (see Table 1 for full structures).




**Scheme 7.** *Reagents and conditions:* a) $Et_2N$, $CH_2Cl_2$; b) **6**, **11**, **25** or **26**, HATU, DIPEA, $CH_2Cl_2$ (20-77% over 2 steps); c) TFA/$H_2O$/thioanisole (90:5:5); d) 1*H*-Pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF (8-42% over 2 steps).

Interestingly, the outcome of the cyclisation of linear precursors for scaffold B was different than for scaffold A (Figure 1A).[10,3,7] For scaffold A, TFA-treatment of the mixture of diastereoisomeric linear precursors led to the formation of two cyclization products, however the cyclization of **32-37** gave two major and either one or two minor peaks as evident by HPLC analysis (see Figure S1). From this point on, the two major guanidinylation products are denoted **a** and **b**, and the minor compounds are denoted **c** and **d**. MS analysis revealed that all these peaks had the expected *m/z* of the cyclized products.

The crude guanidinylation products were purified by semi-preparative RP-HPLC, however separation proved to be difficult due to small differences in retention time between the isomers. Pure diastereoisomers could not be obtained for every analogue and when separation was impossible, mixtures of two isomers were collected and analysed by NMR spectroscopy and mass spectrometry.

### 2.2. NMR Studies on Scaffold B Analogues

In order to assign the configuration of the newly formed chiral centres at C2 and C9a (Figure 1B) for the different cyclization products, the 2D $^1$H ROESY experiment was used. As was the case for scaffold A, the known configuration of C6 was used as a starting point. The configuration of C9a for **45a** and **45b** (Figure 2) could be determined by the presence of strong cross-peaks between H9a and Hβ/Hγ of the $R^2$ side chain in the ROESY spectrum of **45a**, while these were absent in the spectrum of **45b**. Further, the ROESY spectra of both the **a** and **b** fractions showed no cross-peaks between H2 and H9a, indicating that these are *trans* to each other, which is opposite to what was observed for H3/H9a for all analogues based on scaffold A.[3,7] These

observations suggest a (2*S*,6*S*,9a*S*) configuration for **45a** and a (2*R*,6*S*,9a*R*) configuration for **45b** as depicted in Figure 2.

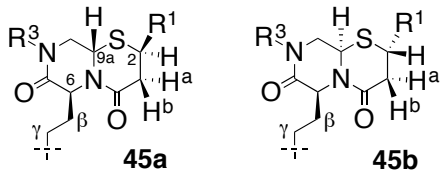



**Figure 2.** Suggested configuration of major isomers **a** and **b**.

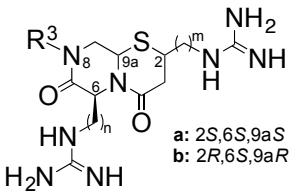
These assignments were further supported by the combination of strong cross-peaks for H9a/H$^b$3 (see Figure 2), a strong cross-peak for H2/H$^a$3, a weaker cross-peak for H2/H$^b$3 and the absence of a H9a/H$^a$3 cross-peak in the ROESY spectrum for **45a**. This suggests that H9a and H$^b$3 are oriented *cis*, while H2 is oriented *trans* to H$^b$3 and *cis* to H$^a$3 for **45a**. For **45b**, the H9a/H3 and H2/H3 correlations were reversed. The NMR data for the **a** and **b** diastereoisomers of **44** and **46-49** were consistent with those of **45a** and **45b**.

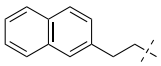
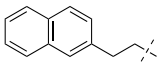
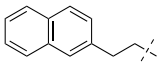
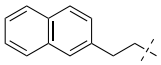
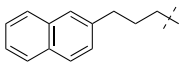
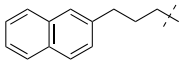
Isomers **c** and **d** could never be isolated in pure form, only as a mixture with varying amounts of isomer **b**. For **48** and **49**, the NMR signals for the two minor products could be partially assigned based on 2D NMR spectra of the mixtures with the **b** isomer (see Table S1 for a comparison of $^1$H chemical shifts for H2, H6 and H9a). The 2D $^1$H ROESY spectra did however not provide a clear-cut answer with regards to the configurational assignment of C2 and C9a, as the spectra were quite crowded and signals overlap in several cases. Based on the 2D NMR and HRMS data, the four products are clearly all bicyclic, and all of them have different chemical shifts for the methine protons of the bicycles. Unfortunately, our present NMR data does not allow for an unequivocal assignment of the configuration of the chiral carbon atoms for isomers **c** and **d**. Interestingly, Grimes et al.[10] noted that in the absence of an R$^1$ substituent, cyclization of linear precursors gave a 90:10 mixture of diastereoisomers, which is comparable to the ratio between major and minor cyclization products in the present work.

### 2.3. Biological Activity for Scaffold B Analogues

The antagonistic potency on human CXCR4 for compounds **44-49** was tested in a functional assay and **45a** showed activity with an $EC_{50}$ value of 61 μM, while its diastereoisomer **45b** was inactive (Table 6). As none of the other synthesised scaffold B analogues were active, this indicates that a three-carbon atom spacer in the R$^1$ side chain is the optimum. On the other hand, it seems detrimental to increase the R$^3$ side chain spacer from two to three carbon atoms in analogues based on scaffold B.

**Table 1.** Structures of compounds **44** – **49** and their antagonistic potency on human CXCR4.




| Compd | m ($R^1$) | n ($R^2$) | $R^3$ | log $EC_{50}$ ± SEM | $EC_{50}$ (μM) | Purity (220 nm) |
|---|---|---|---|---|---|---|
| **44a** | 2 | 3 |  | > −4 | >100 | >95% |
| **44b** | | | | > −4 | >100 | >95% |
| **45a** | 3 | 3 |  | −4.21 ± 0.01 | 61 | >95% |
| **45b** | | | | > −4 | >100 | >95% |
| **46a** | 4 | 3 |  | > −4 | >100 | >95% |
| **46b,c,d** | | | | > −4 | >100 | 8:2 **b**/**c**+**d** |
| **47a** | 5 | 3 |  | > −4 | >100 | >95% |
| **47b** | | | | > −4 | >100 | >95% |
| **48a** | 3 | 3 |  | > −4 | >100 | >95% |
| **48b,c** | | | | > −4 | >100 | 8:2 **b**/**c** |
| **48b,d** | | | | > −4 | >100 | 2:8 **b**/**d** |
| **49a** | 5 | 3 |  | > −4 | >100 | >95% |
| **49b,c** | | | | > −4 | >100 | 7:3 **b**/**c** |
| **49b,d** | | | | > −4 | >100 | 1:9 **b**/**d** |

## 3. Conclusions

A new 2,6,8-trisubstituted bicyclic scaffold has been prepared for the first time, and through 2D NMR studies we have determined the configuration for the major isomer formed. The configuration of the linear precursor directed the stereochemical outcome of the cyclization differently whether the $R^1$ side chain is positioned on C2 (this work) compared to C3 (previous work) in the bicycles. All reports on cyclization to obtain derivatives of 3,6,8-trisubstituted 4,7-dioxo-1-thia-5,8-diaza-bicyclo[4.4.0]decane gave isomers with H3 and H9a in a *cis* relationship, whereas we in this work found that for derivatives of 2,6,8-trisubstituted 4,7-dioxo-1-thia-5,8-diaza-bicyclo[4.4.0]decane, H2 and H9a were *trans* for the major isomers. High

diastereoselectivity in cyclizations to give 3,6,8-trisubstituted bicycles has been reported,[10,3,7] however for the 2,6,8-substitution pattern the diastereoselectivity was lower, with the major and minor isomers formed in an approximately 9:1 ratio. With respect to antagonistic potency toward CXCR4, we found that analogue **45a** displayed comparable activity to our original hit compounds based on the 3,6,8-trisubstituted scaffold and that a three carbon spacer seems to be optimal for the $R^1$ side chain.

## 4. Experimental

4.1. **Chemistry -** All starting materials, reagents and solvents were purchased from Sigma-Aldrich and used as delivered unless stated otherwise. Compounds **4**,[12] **16**[19] and **31**[3] were prepared according to literature procedures and their analytical data were found to be in accordance with those reported. Anhydrous THF was obtained from an anhydrous solvent delivery system (SDS-800 from mBraun) at the Department of Chemistry, University of Bergen. $CH_2Cl_2$ was dried over molecular sieves. For analysis by thin layer chromatography (TLC), aluminium sheets coated with Merck KGaA silica gel (60 $F_{254}$) were used. The TLC plates were visualized using either ultraviolet light or by immersion in a solution of 2% ninhydrin in ethanol supplemented with 10 drops of concentrated sulfuric acid per 100 mL solution, followed by heating. Purification by flash column chromatography was performed on Merck KGaA Kieselgel (230 – 400 mesh). All final compounds were purified by semi-preparative RP-HPLC eluting with mixtures of acetonitrile and $H_2O$ (both containing 0.1% TFA). Fractions of equal purity were pooled and lyophilized. All tested compounds were analysed by RP-HPLC and the purity is specified for each compound below. NMR spectra were obtained on a Bruker Biospin DPX400, a Bruker Biospin AV500, a Bruker Biospin AV600 or a Bruker Biospin 850SB. High resolution mass spectra were obtained on a JEOL AccuTOF™ JMS T100LC which was operated in ESI mode.

4.1.1. *tert*-Butyl 2-oxo-4-(tritylthio)piperidine-1-carboxylate (**5**)

To a stirred mixture of *N*-Boc-protected α,β-unsaturated lactam **4** (339 mg, 1.55 mmol) and triphenylmethanethiol (639 mg, 2.33 mmol) in dry $CH_2Cl_2$ (9.0 mL) was added triethylamine (325 µL, 2.33 mmol). The reaction mixture was stirred at room temperature for 29 h, diluted with $CH_2Cl_2$ (25 mL) and washed with saturated NaCl (20 mL). The solvent was removed under reduced pressure to give the crude product (1.27 g). Purification by automated column chromatography on silica gel (hexanes/EtOAc, 8:2) gave the title compound (503 mg, 68%). $R_f$ (hexanes/EtOAc, 8:2) = 0.21; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.50 – 7.43 (m, 6H), 7.33 – 7.27 (m, 6H), 7.25 – 7.19 (m, 3H), 3.60 (dt, $J$ = 12.9, 5.0 Hz, 1H), 3.17 – 3.08 (m, 1H),

2.60 – 2.50 (m, 2H), 2.44 – 2.34 (m, 1H), 1.47 (s, 9H), 1.46 – 1.38 (m, 2H); $^{13}$C NMR (100.6 MHz, $CDCl_3$): $\delta$ = 169.1, 152.4, 144.6, 129.5, 128.2, 127.0, 83.2, 68.0, 44.9, 42.1, 37.8, 30.6, 28.0; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{29}H_{31}NO_3SNa^+$: 496.1917; found: 496.1924.

4.1.2. 5-((*tert*-Butoxycarbonyl)amino)-3-(tritylthio)pentanoic acid (**6**)

To a stirred solution of *tert*-butyl 2-oxo-4-(tritylthio)piperidine-1-carboxylate (approximately 500 mg, 1.05 mmol) in THF (5.0 mL) was added LiOH (1 M, 2.0 mL, 2.0 mmol). The reaction mixture was stirred at room temperature and monitored by TLC. After 1 h the starting material was consumed completely and the solvent was removed under reduced pressure. The aqueous residue was acidified to pH ~4 with 10% citric acid and extracted with $CH_2Cl_2$ (20 mL, then 3 x 5 mL). The combined organic layer was washed with saturated NaCl (40 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product (477 mg). Purification by flash column chromatography on silica gel ($CH_2Cl_2$/MeOH, 95:5) gave the title compound as a colorless oil (424 mg, 82%). $R_f$ ($CH_2Cl_2$/MeOH, 95:5) = 0.20; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.55 – 7.51 (m, 6H), 7.29 (t, *J* = 7.7 Hz, 6H), 7.22 – 7.18 (m, 3H), 4.25 (bs, 1H), 3.16 – 2.96 (m, 2H), 2.60 (s, 1H), 2.34 – 2.11 (m, 2H), 1.72 – 1.62 (m, 1H), 1.56 – 1.47 (m, 1H), 1.41 (s, 9H); $^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ = 176.5, 156.0, 144.8, 129.6, 128.1, 126.9, 79.3, 67.8, 39.7, 38.4, 37.8, 35.0, 28.5; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{29}H_{33}NO_4SNa^+$: 514.2023; found: 514.2030.

4.1.3. *tert*-Butyl 2-oxo-3-(phenylselanyl)azepane-1-carboxylate (**8**)

Lithium bis(trimethylsilyl)amide (1.0 M in THF, 1.80 mL, 1.80 mmol) was added drop-wise to a stirred solution of *N*-Boc 6-aminohexanoic acid lactam (0.30 mL, 1.46 mmol) in dry THF (3 mL) at -78 °C. The solution was stirred for 1 h before phenylselenyl chloride (0.36 g, 1.90 mmol) in dry THF (3 mL) was added drop-wise. After 2 h of stirring at -78 °C the reaction mixture was allowed to warm to room temperature before HCl (1.0 M, 10 mL) was added to quench the reaction. The reaction mixture was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with $NaHCO_3$ (10%, 30 mL) and saturated NaCl (30 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give the crude product (624 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 9:1) gave the title compound as a white solid (462 mg, 86%). $R_f$ (hexanes/EtOAc, 9:1) = 0.16; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.62 – 7.58 (m, 2H), 7.31 – 7.26 (m, 3H), 4.29 (dd, *J* = 9.4, 2.5 Hz, 1H), 4.10 – 4.02 (m, 1H), 3.75 – 3.67 (m, 1H), 2.21 – 2.11 (m, 1H), 2.09 – 1.97 (m, 1H), 1.89 – 1.79 (m, 1H), 1.76 – 1.65 (m, 2H), 1.64 – 1.55 (m, 1H), 1.52 (s, 9H); $^{13}$C NMR (100.6 MHz, $CDCl_3$): $\delta$ = 173.7, 153.2, 134.6, 129.6, 129.3,

128.1, 83.3, 51.1, 45.7, 31.6, 28.4, 28.3, 28.1; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{17}H_{23}NO_3SeNa^+$: 392.0735; found: 392.0743.

4.1.4. *tert*-Butyl 2-oxo-4-(tritylthio)azepane-1-carboxylate (**10**)

A stirred solution of **8** (77 mg, 0.21 mmol) in THF (0.5 mL) was cooled to 0 °C and $H_2O_2$ (30%, 50 µL, 0.44 mmol) was added drop-wise. The reaction mixture was stirred at 0 °C for 17 minutes and then warmed up to room temperature over 20 minutes. The reaction mixture was separated between $CH_2Cl_2$ (5 mL) and saturated $NaHCO_3$ (5 mL). The aqueous layer was extracted with $CH_2Cl_2$ (5 mL) and the combined organic layers were washed with saturated NaCl and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the crude *N*-Boc (*Z*) 6-aminohex-2-enoic acid lactam (**9**) as yellow oil (58 mg). The crude product (58 mg) and triphenylmethanethiol (156 mg, 0.56 mmol) were dissolved in anhydrous DMF (3 mL). Triethylamine (80 µl, 0.58 mmol) was added and the reaction mixture was stirred for 18 h. The solvent was removed under reduced pressure to give the crude product (290 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 4:1) gave the title compound as white foam (68 mg, 66% over two steps). $R_f$ (hexanes/EtOAc, 4:1) = 0.19; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.51 – 7.46 (m. 6H), 7.31 – 7.25 (m, 6H), 7.22 – 7.17 (m, 3H), 3.68 – 3.54 (m, 2H), 2.72 – 2.54 (m, 3H), 1.88 – 1.76 (m, 1H), 1.48 (s, 9H), 1.46 – 1.30 (m, 3H); $^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ = 172.3, 152.6, 144.8, 129.7, 128.1, 126.9, 82.9, 68.3, 46.3, 45.3, 39.9, 35.7, 28.2, 26.2; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{33}NO_3SNa^+$: 510.2073; found: 510.2078; *m/z* $[2M+Na]^+$ calcd for $C_{60}H_{66}N_2O_6S_2Na^+$: 997.4255; found: 997.4283.

4.1.5. 6-(*tert*-Butoxycarbonylamino)-3-(tritylthio)hexanoic acid (**11**)

To a stirred solution of **10** (118 mg, 0.24 mmol) in THF (1 mL) was added LiOH (1.0 M, 0.73 mL, 0.73 mmol). The reaction was monitored by TLC and all starting material was consumed after 1 h. The solvent was removed under reduced pressure and the aqueous residue was acidified to pH 4 with 10% citric acid followed by extraction with $CH_2Cl_2$ (20 mL, then 3 x 5 mL). The organic layers were combined, washed with saturated NaCl (25 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product (117 mg). Purification by flash column chromatography on silica gel ($CH_2Cl_2$/MeOH, 95:5) gave the title compound as a colorless oil (87 mg, 71%). $R_f$ ($CH_2Cl_2$/MeOH, 95:5) = 0.23; $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.55 – 7.52 (m, 6H), 7.31 – 7.27 (m, 6H), 7.23 – 7.19 (m, 3H), 4.57 (bs, 1H), 3.00 – 2.86 (m, 2H), 2.70 – 2.63 (m, 1H), 2.24 (dd, *J* = 16.1, 9.4 Hz, 1H), 2.04 (d, *J* = 15.9 Hz, 1H), 1.44 (s, 9 H), 1.41 – 1.31 (m, 4H); $^{13}$C NMR

(125.8 MHz, $CDCl_3$): $\delta$ = 175.5, 156.0, 144.9, 129.6, 128.1, 126.8, 79.2, 67.6, 40.6, 40.3, 39.6, 31.8, 28.5, 26.4; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{35}NO_4SNa^+$: 528.2179; found: 528.2187.

4.1.6. *tert*-Butyl (6-oxohexyl)carbamate (**18**)

To a stirred solution of crude **17** (1.92 g) in dry $CH_2Cl_2$ (10 mL) at 0 °C was added Dess-Martin periodinane (3.74 g, 8.81 mmol). The reaction mixture was stirred at room temperature for 25 h. Slow addition of a mixture of saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1, 10 mL) and stirred for another 30 minutes. The two layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with $H_2O$ (2 x 20 mL) and saturated NaCl (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as light yellow oil (2.03 g). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 6:4) gave the title compound as colorless liquid (661 mg, 37% over 2 steps). $R_f$ (hexanes/EtOAc, 6:4) = 0.38; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 9.76 (t, *J* = 1.8 Hz, 1H), 4.58 (bs, 1H), 3.16 – 3.06 (m, 2H), 2.43 (td, *J* = 7.3, 1.6 Hz, 2H), 1.65 (p, *J* = 7.5 Hz, 2H), 1.54 – 1.47 (m, 2H), 1.44 (s, 9H), 1.39 – 1.32 (m, 2H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 202.6, 156.1, 79.2, 43.8, 40.4, 30.0, 28.5, 26.4, 21.8; HRMS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{11}H_{21}NO_3Na^+$: 238.1414; found: 238.1418; *m/z* $[2M + Na]^+$ calcd for $C_{22}H_{42}N_2O_6Na^+$: 453.2935; found: 453.2930.

4.1.7. Methyl (*E*)-7-((di-*tert*butoxycarbonyl)amino)hept-2-enoate (**19**)

To a stirred solution of **16** (195 mg, 0.64 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added Dess-Martin periodinane (319 mg, 0.75 mmol). The reaction mixture was stirred at 0 °C for 2 h. Saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1, 10 mL) was added drop wise to the reaction mixture and stirring continued for 30 minutes. The two layers were separated and the aqueous layer was extracted with diethyl ether (4 x 10 mL). The organic layers were combined, washed with $H_2O$ (3 x 20 mL) and saturated NaCl (20 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product (193 mg), which was used in the subsequent step without purification. To a stirred solution of the crude aldehyde (193 mg) made in the previous step in dry THF (5 mL) was added methyl (triphenylphosphoranylidene)acetate (454 mg, 1.36 mmol). The reaction mixture was stirred at 60 °C for 24 h. The solvent was removed under reduced pressure to give the crude product as an orange solid (620 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 8:2) gave the title compound (187 mg, 81%). $R_f$ (hexanes/EtOAc, 8:2) = 0.34; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.95 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.82 (dt, *J* = 15.6, 1.5 Hz, 1H), 3.72 (s, 3H), 3.57 (t, 7.3 Hz, 2H), 2.25 – 2.20 (m, 2H), 1.64 – 1.56 (m, 2H), 1.50 (s, 18H), 1.48 – 1.42 (m, 2H); $^{13}$C NMR (125.8

MHz, $CDCl_3$): $\delta$ = 167.2, 152.9, 149.2, 121.3, 82.3, 51.6, 46.2, 32.0, 28.7, 28.2, 25.4; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{18}H_{31}NO_6Na^+$: 380.2044; found: 380.2047.

4.1.8. Methyl (*E*)-8-((*tert*-butoxycarbonyl)amino)oct-2-enoate (**20**)

Boc-protected aldehyde **18** (640 mg, 2.97 mmol) was dissolved in dry THF (15 mL) and methyl(triphenylphosphoranylidene)acetate (1.40 g, 4.17 mmol) was added. The mixture was stirred at 60 °C for 21 h under argon atmosphere. The solvent was removed under reduced pressure to give the crude product as a yellow solid (1.95 g). Purification by flash chromatography on silica gel (hexanes/EtOAc 7:3) gave the title compound as a colorless liquid (456 mg, 57%). $R_f$ (hexanes/EtOAc, 7:3) = 0.37; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.95 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.82 (dt, *J* = 15.7, 1.5 Hz, 1H), 4.59 (bs, 1H), 3.72 (s, 3H), 3.17 – 3.01 (m, 2H), 2.21 (qd, *J* = 7.2, 1.6 Hz, 2H), 1.52 – 1.46 (m, 4H), 1.44 (s, 9H), 1.38 – 1.30 (m, 2H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 167.2, 156.1, 149.4, 121.2, 79.1, 51.5, 40.5, 32.1, 30.0, 28.5, 27.8, 26.4; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{14}H_{25}NO_4Na^+$: 294.1676; found: 294.1683.

4.1.9. 1,1,1,3,3,3-hexafluoropropan-2-yl (*E*)-7-((di-*tert*-butoxycarbonyl)amino)hept-2-enoate (**21**)

LiOH (1 M, 1.6 mL, 1.6 mmol) was added to a stirred solution of unsaturated methyl ester **19** (187 mg, 0.52 mmol) in THF (2 mL). The reaction mixture was stirred for room temperature for 11.5 h. The solvent was removed under reduced pressure and the pH of the aqueous residue was acidified to pH ~ 4 with 10% citric acid. The mixture was extracted with $CH_2Cl_2$ (4 x 10 mL) and the combined organic layer was washed with saturated NaCl (25 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product (170 mg). Analysis by ESI-MS showed incomplete conversion of starting material, therefore the crude product (170 mg) was dissolved in THF (2 mL) and treated with LiOH (1 M, 6 mL, 6 mmol). The reaction mixture was stirred at room temperature for 20 h. The solvent was removed under reduced pressure and the pH of the aqueous residue was acidified to pH ~ 4 with 10% citric acid. The mixture was extracted with $CH_2Cl_2$ (4 x 10 mL), the combined organic layer was washed with saturated NaCl (25 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product (151 mg), which was used in the next step without purification. To a stirred solution of crude carboxylic acid (151 mg) in dry $CH_2Cl_2$ (2 mL) was added DMAP (24 mg, 0.19 mmol), DIC (80 μL, 0.52 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol (60 μL, 0.57 mmol). The reaction mixture was stirred at room temperature for 21.5 h and then diluted with $CH_2Cl_2$ (15 mL), washed with saturated $NH_4Cl$ (10 mL) and saturated NaCl (10 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give the crude product (408 mg). Purification by flash column chromatography on silica

gel (hexanes/EtOAc, 9:1) gave the title compound (160 mg, 62% over 2 steps). $R_f$ (hexanes/EtOAc, 9:1) = 0.28; $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.20 (dt, $J$ = 15.6, 6.9 Hz, 1H), 5.94 (dt, $J$ = 15.7, 1.6 Hz, 1H), 5.82 (sep, $J$ = 6.1 Hz, 1H), 3.58 (t, $J$ = 7.3 Hz, 2H), 2.34 – 2.29 (m, 2H), 1.64 – 1.58 (m, 2H), 1.54 – 1.45 (m, 20H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 162.9, 154.7, 152.8, 120.6 (q, $^1J_{CF}$ = 283.1 Hz), 118.3, 82.4, 66.4 (sep, $^2J_{CF}$ = 34.5 Hz), 46.0, 32.3, 28.5, 28.2, 25.0; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{20}H_{29}F_6NO_6Na^+$: 516.1791; found: 516.1800.

4.1.10. 1,1,1,3,3,3-Hexafluoropropan-2-yl (*E*)-8-((*tert*-butoxycarbonyl)amino)-oct-2-enoate (**22**)

Methyl ester **20** (385 mg, 1.42 mmol) was dissolved in THF (10 mL) and LiOH (1 M, 4.50 mL, 4.50 mmol) was added. The mixture was stirred at room temperature for 16.5 h. The solvent was removed under reduced pressure upon completion and the aqueous residue was acidified to pH 4 with 10% citric acid and extracted with $CH_2Cl_2$ (3 x 40 mL). The combined organic layers were washed with saturated NaCl (3 x 50 mL) and dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as an orange solid (334 mg), which was used in the next step without purification.

To a stirred solution of crude carboxylic acid (324 mg, 1.26 mmol) in dry $CH_2Cl_2$ (3 mL) was added 1,1,1,3,3,3-hexafluoro-2-propanol (0.16 mL, 1.52 mmol), DMAP (63 mg, 0.52 mmol) and DIC (0.25 mL, 1.61 mmol). The reaction mixture was stirred for 20 h and then diluted with $CH_2Cl_2$ (25 mL), washed with saturated $NH_4Cl$ (20 mL) and saturated NaCl (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to yield the crude product as a red solid (622 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 1:1) gave the title compound as yellow oil (315 mg, 61%). $R_f$ (hexanes/EtOAc, 1:1) = 0.14; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.20 (dt, $J$ = 15.7, 6.8 Hz, 1H), 5.93 (dt, $J$ = 15.7, 1.6 Hz, 1H), 5.82 (sep, $J$ = 6.1 Hz, 1H), 4.51 (s, 1H), 3.15 – 3.09 (m, 2H), 2.28 (dq, $J$ = 7.1, 1.6 Hz, 2H), 1.54 – 1.47 (m, 4H), 1.44 (s, 9H), 1.39 – 1.34 (m, 2H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 163.0, 156.1, 154.9, 120.7 (q, $^1J_{CF}$ = 282.1 Hz), 118.2, 79.3, 66.5 (sep, $^2J_{CF}$ = 34.2 Hz), 40.5, 32.7, 30.1, 28.6, 27.5, 26.5; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{16}H_{23}F_6NO_4Na^+$: 430.1424, found: 430.1428.

4.1.11. 1,1,1,3,3,3-Hexafluoropropan-2-yl 7-((di-*tert*-butoxycarbonyl)amino)-3-(tritylthio)heptanoate (**23**)

To a stirred solution of HFIP-ester **21** (124 mg, 0.25 mmol) in dry $CH_2Cl_2$ (5 mL) was added $Et_3N$ (0.14 mL, 1.00 mmol) and triphenylmethanethiol (339 mg, 1.23 mmol). The reaction mixture was stirred at room temperature for 69 h and then diluted with $CH_2Cl_2$ (30 mL), washed with $H_2O$ (40 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product as yellow oil (427 mg). Purification by flash column

chromatography on silica gel (hexanes/EtOAc, 95:5) gave the title compound (101 mg, 52%). $R_f$ (hexanes/EtOAc, 95:5) = 0.12; $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.54 – 7.51 (m, 6H), 7.30 – 7.27 (m, 6H), 7.22 – 7.19 (m, 3H), 5.61 (sep, $J$ = 6.1 Hz, 1H), 3.51 (t, $J$ = 7.5 Hz, 2H), 2.70 – 2.65 (m, 1H), 2.20 (dd, $J$ = 16.7, 9.8 Hz, 1H), 1.80 (dd, $J$ = 16.8, 3.4 Hz, 1H), 1.59 – 1.44 (m, 4H), 1.50 (s, 18H), 1.41 – 1.25 (m, 2H); $^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ = 168.3, 152.8, 144.7, 129.5, 128.2, 126.9, 120.4 (q, $^1J_{CF}$ = 282.2 Hz), 82.2, 67.7, 66.1 (sep, $^2J_{CF}$ = 34.6 Hz), 46.2, 40.7, 38.4, 34.3, 29.1, 28.2, 23.6; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{39}H_{45}F_6NO_6SNa^+$: 792.2764; found: 792.2767.

4.1.12. 1,1,1,3,3,3-Hexafluoropropan-2-yl 8-((*tert*-butoxycarbonyl)amino)-3-(tritylthio)octanoate (**24**)

To a stirred solution of unsaturated HFIP ester **22** (315 mg, 0.77 mmol) in dry $CH_2Cl_2$ (5 mL) was added triphenylmethanethiol (641 mg, 2.32 mmol) and $Et_3N$ (0.32 mL, 2.39 mmol). The reaction mixture was stirred at room temperature for 48 h and then diluted with $CH_2Cl_2$ (30 mL) and washed with $H_2O$ (40 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as yellow oil (958 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 9:1) gave the title compound as yellow foam (483 mg, 91%). $R_f$ (hexanes/EtOAc, 9:1) = 0.19; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.53 – 7.51 (m, 6H), 7.30 – 7.27 (m, 6H), 7.22 – 7.19 (m, 3H), 5.61 (sep, $J$ = 6.1 Hz, 1H), 4.47 (bs, 1H), 3.15 – 3.01 (m, 2H), 2.69 – 2.66 (m, 1H), 2.22 (dd, $J$ = 16.6, 9.9 Hz, 1H), 1.83 (dd, $J$ = 16.6, 3.4 Hz, 1H), 1.55 – 1.48 (m, 2H), 1.45 (s, 9H), 1.44 – 1.31 (m, 4H), 1.23 – 1.16 (m, 2H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 168.3, 156.1, 144.7, 129.6, 128.2, 126.9, 120.4 (q, $^1J_{CF}$ = 282.5 Hz), 79.2, 67.7, 66.2 (sep, $^2J_{CF}$ = 34.8 Hz), 40.7 (2C), 38.5, 34.6, 30.0, 28.6, 26.8, 26.0; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{35}H_{39}F_6NO_4SNa^+$: 706.2396; found: 706.2402.

4.1.13. 7-((Di-*tert*-butoxycarbonyl)amino)-3-(tritylthio)heptanoic acid (**25**)

To a stirred solution of Michael product **23** (448 mg, 0.58 mmol) in THF (2 mL) was added LiOH (1 M, 4 mL, 4 mmol). The reaction mixture was stirred at room temperature for 17 h. The solvent was removed under reduced pressure and the pH of the aqueous residue was acidified to pH ~ 4 with 10% citric acid. The mixture was extracted with $CH_2Cl_2$ (4 x 10 mL) and the combined organic layer was washed with saturated NaCl (25 mL), dried over anhydrous $MgSO_4$ and concentrated to give the crude product as yellow oil (321 mg, 33%). Purification by flash column chromatography on silica gel ($CH_2Cl_2$/MeOH, 95:5) gave the title compound as a colorless oil (116 mg). $R_f$ ($CH_2Cl_2$/MeOH, 95:5) = 0.30; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ =

7.53 – 7.50 (m, 6H), 7.29 – 7.25 (m, 6H), 7.21 – 7.17 (m, 3H), 3.48 (t, $J$ = 7.4 Hz, 2H), 2.68 – 2.62 (m, 1H), 2.17 (dd, $J$ = 16.4, 9.0 Hz, 1H), 1.93 (16.4, 4.2 Hz, 1H), 1.49 (s, 18H), 1.46 – 1.35 (m, 4H), 1.34 – 1.21 (m, 2H); $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 176.8, 152.8, 144.9, 129.7, 128.1, 126.8, 82.3, 67.7, 46.4, 41.0, 39.2, 34.5, 29.1, 28.2, 23.6; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{36}H_{45}NO_6SNa^+$: 642.2860; found: 642.2868.

4.1.14. 8-((*tert*-Butoxycarbonyl)amino)-3-(tritylthio)octanoic acid **(26**)

To a stirred solution of HFIP ester **24** (201 mg, 0.30 mmol) in THF (10 mL) was added LiOH (1 M, 2.0 mL, 2.0 mmol). The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue was acidified to pH 4 with 10% citric acid. The mixture was extracted with $CH_2Cl_2$ (4 x 25 mL) and the combined organic layer was washed with saturated NaCl (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as yellow foam (309 mg). Purification by flash column chromatography on silica gel ($CH_2Cl_2$/MeOH, 95:5) gave the title compound as yellow foam (197 mg, 56%). Retains $CH_2Cl_2$. $R_f$ ($CH_2Cl_2$/MeOH, 95:5) = 0.28; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 12.09 (s, 1H), 7.44 – 7.42 (m, 6H), 7.36 – 7.31 (m, 6H), 7.25 – 7.22 (m, 3H), 6.73 (t, $J$ = 5.4 Hz, 1H), 2.84 (q, $J$ = 6.6 Hz, 2H), 2.57 – 2.52 (m, 1H), 2.16 (dd, $J$ = 16.3, 9.0 Hz, 1H), 1.87 (dd, $J$ = 16.3, 4.2 Hz, 1H), 1.38 (s, 9H), 1.29 – 1.23 (m, 4H), 1.19 – 1.07 (m, 3H), 1.03 – 0.98 (m, 2H); $^{13}$C NMR (125.8 MHz, DMSO-$d_6$): $\delta$ = 172.2, 155.5, 144.6, 129.1, 128.0, 126.6, 77.2, 66.9, 40.8, 39.8 (HSQC), 39.1 (HSQC), 34.1, 29.3, 28.3, 26.2, 25.3; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{32}H_{39}NO_4SNa^+$: 556.2492; found: 556.2499.

4.1.15. *tert*-Butyl (3-(naphthalene-2-yl)propyl)carbamate (**28**)

*N*-Boc allylamine **27** (1.02 g, 6.5 mmol) was dissolved in dry THF (6.0 mL) and 9-BBN (0.5 M in THF, 20.0 mL, 10.0 mmol) was added. The resulting mixture was stirred at room temperature for 2.5 h before 2-bromonaphthalene (1.35 g, 6.5 mmol), $Pd(dppf)_2Cl_2 \cdot CH_2Cl_2$ (308 mg, 0.4 mmol) and NaOH (1 M, 10.0 mL, 10.0 mmol) were added. The reaction mixture was stirred at room temperature for 3 h, then degassed and additional $Pd(dppf)_2Cl_2 \cdot CH_2Cl_2$ (55 mg, 0.07 mmol) was added. The reaction mixture was stirred for 14 h at room temperature before saturated $NH_4Cl$ (40 mL) was added. The resulting mixture was extracted with EtOAc (4 x 25 ml) and the combined organic layer was dried over $MgSO_4$, filtered and concentrated to yield the crude product as brown oil (3.28 g). Purification by flash column chromatography on silica gel (hexanes/EtOAc 8.5:1.5 and second column using hexanes/EtOAc 8:2, respectively) gave a white solid (1.36 g), which still contained minor impurities ($^1$H NMR). To a solution of the solid material (818 mg out of 1.36

g) in THF (20 mL), NaOH (15%, 5.0 mL) and $H_2O_2$ (30%, 10.0 mL) were added and the resulting mixture was stirred at 0 °C for 2 h. Dilution with diethyl ether (20 mL) followed by washing with saturated $NaHCO_3$ (20 mL) and saturated NaCl (20 mL) before the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to give a yellow oil (785 mg). Purification by flash column chromatography on silica gel (hexanes/EtOAc, 8.5:1.5) gave the title compound (774 mg, 70%). $R_f$ (hexanes/EtOAc, 8.5:1.5) = 0.24; $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.82 – 7.76 (m, 3H), 7.62 (s, 1H), 7.47 – 7.41 (m, 2H), 7.32 (dd, $J$ = 8.4, 1.2 Hz, 1H), 4.56 (bs, 1H), 3.24 – 3.15 (m, 2H), 2.81 (t, $J$ = 7.8 Hz, 2H), 1.90 (p, $J$ = 7.5 Hz, 2H), 1.46 (s, 9H); $^{13}$C NMR (150.9 MHz, $CDCl_3$): $\delta$ = 156.1, 139.2, 133.7, 132.2, 128.1, 127.7, 127.5, 127.3, 126.5, 126.1, 125.3, 79.3, 40.5, 33.4, 31.8, 28.6; HRMS (ESI): $m/z$ $[M+Na]^+$ calcd for $C_{18}H_{23}NO_2Na^+$: 308.1621; found: 308.1628.

4.1.16. *N*-(2,2-Dimethoxyethyl)-3-(naphthalen-2-yl)propan-1-amine (**29**)

To a stirred solution of **28** (321 mg, 1.12 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added TFA (4.0 mL). The reaction mixture was stirred at room temperature for 2.5 h, after which the solvent was removed under reduced pressure. The residue was suspended in 10% NaOH (15 mL) and extracted with $CH_2Cl_2$ (4 x 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as brown oil (192 mg), which was used in the next step without purification. To a stirred solution of crude 3-(naphthalene-2-yl)propan-1-amine (192 mg) in anhydrous DMF (5 mL) was added bromoacetaldehyde dimethyl acetal (0.18 mL, 1.52 mmol) and $K_2CO_3$ (0.145 g, 1.05 mmol). The reaction mixture was stirred for 27.5 h at 80 °C. The mixture was diluted with $H_2O$ (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with saturated NaCl (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as yellow oil (572 mg). Purification by flash column chromatography on silica gel ($CH_2Cl_2$/MeOH/$NH_4OH$, 95:4.5:0.5) gave the title compound as pale yellow oil (96 mg, 31%). $R_f$ ($CH_2Cl_2$/MeOH/$NH_4OH$, 95:4.5:0.5) = 0.31; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.82 – 7.74 (m, 3H), 7.61 (s, 1H), 7.47 – 7.38 (m, 2H), 7.33 (dd, $J$ = 8.4, 1.7 Hz, 1H), 4.47 (t, $J$ = 5.5 Hz, 1H), 3.37 (s, 6H), 2.81 (t, $J$ = 7.5 Hz, 2H), 2.73 (d, $J$ = 5.5 Hz, 2H), 2.69 (t, $J$ = 7.2 Hz, 2H), 1.92 (p, $J$ = 7.6 Hz, 2H), 1.65 (bs, 1H); $^{13}$C NMR (100.6 MHz, $CDCl_3$): $\delta$ = 139.6, 133.7, 132.1, 128.0, 127.7, 127.5, 127.4, 126.4, 126.0, 125.2, 103.9, 54.2, 51.2, 49.5, 33.8, 31.6; HRMS (ESI): $m/z$ $[M+H]^+$ calcd for $C_{17}H_{24}NO_2^+$: 274.1802; found: 274.1809.

4.1.17. (9*H*-Fluoren-9-yl)methyl (*S*)-(1-((2,2-dimethoxyethyl)(3-(naphthalen-2- yl)propyl)amino)-1-oxo-5-

(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (**30**)

To a stirred solution of **29** (96 mg, 0.35 mmol) in dry $CH_2Cl_2$ (2 mL) was added Fmoc-Arg(Pbf)-OH (244 mg, 0.38 mmol), HATU (136 mg, 0.36 mmol) and DIPEA (0.20 mL, 1.15 mmol). The resulting mixture was stirred for 25 h at room temperature. The solvent was removed under reduced pressure and the residue was partitioned between $H_2O$ (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layer was washed with 1 M $KHSO_4$ (20 mL), $H_2O$ (20 mL), saturated $NaHCO_3$ (20 mL) and saturated NaCl (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product (341 mg). Purification by flash column chromatography on silica gel (EtOAc/hexanes, 8:2) gave the title compound (250 mg, 79%). $R_f$ (EtOAc/hexanes, 8:2) = 0.10; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{51}H_{62}N_5O_8S^+$: 904.4315; found: 904.4314; *m*/*z* $[M+Na]^+$ calcd for $C_{51}H_{61}N_5O_8SNa^+$: 926.4133; found: 926.4139.

4.1.18. General protocol A: Fmoc-deprotection

To a stirred solution of the Fmoc-protected amine (0.06 M in $CH_2Cl_2$) was added $Et_2NH$ (50 equiv) and the resulting mixture was stirred at room temperature for 2-3 h (monitored by TLC). The solvent was removed under reduced pressure and the residue was used in the next step without purification.

4.1.19. General protocol B: Formation of linear precursors

HATU (165 mg, 0.43 mmol) and DIPEA (0.19 mL, 1.1 mmol) were added to a stirred solution the carboxylic acid (**6**, **11**, **25** or **26**) carrying the $R^1$ side chain (0.36 mmol) in dry $CH_2Cl_2$ (2 mL) and the reaction mixture was stirred at room temperature for 20 minutes. Fmoc-deprotected amine **30** or **31** (see general protocol A, 0.56 mmol) in dry $CH_2Cl_2$ (12 mL) was then added and stirring was continued for 24 h. The solvent was evaporated, and the resulting red residue was partitioned between $H_2O$ (10 mL) and EtOAc (10 mL). The aqueous layer was further extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with 1 M $KHSO_4$ (20 mL), $H_2O$ (20 mL), saturated $NaHCO_3$ (20 mL) and saturated NaCl (20 mL). The organic layer was further dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product.

4.1.20. *tert*-Butyl (5-(((*S*)-1-((2,2-dimethoxyethyl)(2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)pentan-2-yl)amino)-5-oxo-3-(tritylthio)pentyl)carbamate (**32**)

The title compound was prepared from carboxylic acid **6** (177 mg, 0.36 mmol) which was dissolved in DMF (2 mL) and Fmoc-deprotected amine **31** (see general protocol B, 0.56 mmol) following general protocol A. The crude product (red foam, 510 mg) was purified by flash column chromatography on silica gel (EtOAc/hexane; 7.5:2.5 to 9.5:0.5 followed by EtOAc) to give an inseparable mixture of diastereoisomers of **32** (174 mg, 42%). $R_f$ (EtOAc) = 0.7; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{64}H_{81}N_6O_9S_2^+$: 1141.5501; found: 1141.5502; *m*/*z* $[M+Na]^+$ calcd for $C_{64}H_{80}N_6O_9S_2Na^+$: 1163.5320; found: 1163.5314.

4.1.21. *tert*-Butyl (6-(((*S*)-1-((2,2-dimethoxyethyl)(2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxo-4-(tritylthio)hexyl)carbamate (**33**)

The title compound was prepared from carboxylic acid **11** (160 mg, 0.32 mmol) and Fmoc-deprotected amine **31** (see general protocol B, 0.45 mmol) following general protocol A. The crude product (red foam, 654 mg) was purified by flash column chromatography on silica gel (EtOAc/hexane; 8:2) to give an inseparable mixture of diastereoisomers of **33** (216 mg, 59%). $R_f$ (EtOAc/hexanes, 8:2) = 0.19; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{65}H_{83}N_6O_9S_2^+$: 1155.5658; found: 1155.5664; *m*/*z* $[M+Na]^+$ calcd for $C_{65}H_{82}N_6O_9S_2Na^+$: 1177.5477; found: 1177.5529.

4.1.22. Di-*tert*-butyl (7-(((*S*)-1-((2,2-dimethoxyethyl)(2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)pentan-2-yl)amino)-7-oxo-5-(tritylthio)heptyl)carbamate (**34**)

The title compound was prepared from carboxylic acid **25** (116 mg, 0.19 mmol) and Fmoc-deprotected amine **31** (see general protocol B, 0.44 mmol) following general protocol A. The crude product (red oil, 444 mg) was purified by flash column chromatography on silica gel (EtOAc/hexanes, 8:2) to give an inseparable mixture of diastereoisomers of **34** as a colorless oil (47 mg, 20%). $R_f$ (EtOAc/hexanes, 8:2) = 0.57; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{71}H_{92}N_6O_{11}S_2Na^+$: 1291.6158; found: 1291.6166.

4.1.23. *tert*-Butyl (8-(((*S*)-1-((2,2-dimethoxyethyl)(2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)pentan-2-yl)amino)-8-oxo-6-(tritylthio)octyl)carbamate (**35**)

The title compound was prepared from carboxylic acid **26** (105 mg, 0.20 mmol) and Fmoc-deprotected amine **31** (see general protocol B, 0.10 mmol) following general protocol A. The crude product (205 mg) was purified by flash column chromatography on silica gel (EtOAc/hexanes, 9:1) to give an inseparable mixture of diastereoisomers of **35** as a colorless oil (80 mg, 68%). $R_f$ (EtOAc/hexanes, 9:1) = 0.25; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{67}H_{86}N_6O_9S_2Na^+$: 1205.5790; found: 1205.5796.

4.1.24. *tert*-Butyl (6-(((*S*)-1-((2,2-dimethoxyethyl)(3-(naphthalen-2-yl)propyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)pentan-2-yl)amino)-6-oxo-4-(tritylthio)hexyl)carbamate (**36**)

The title compound was prepared from carboxylic acid **11** (166 mg, 0.33 mmol) and Fmoc-deprotected amine **30** (see general protocol B, 0.10 mmol) following general protocol A. The crude product (273 mg) was purified by flash column chromatography on silica gel (EtOAc/hexane, 9:1) to give an inseparable mixture of diastereoisomers of **36** as a colorless oil (90 mg, 73%). $R_f$ (EtOAc/hexanes, 9:1) = 0.35; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{66}H_{84}N_6O_9S_2Na^+$: 1191.5633; found: 1191.5640.

4.1.25. *tert*-Butyl (8-(((*S*)-1-((2,2-dimethoxyethyl)(3-(naphthalen-2-yl)propyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)pentan-2-yl)amino)-8-oxo-6-(tritylthio)octyl)carbamate (**37**)

The title compound was prepared from carboxylic acid **27** (93 mg, 0.17 mmol) and Fmoc-deprotected amine **30** (see general protocol B, 0.13 mmol) following general protocol A. The crude product (238 mg) was purified by flash column chromatography on silica gel (EtOAc/hexane, 8:2) to give an inseparable mixture of diastereoisomers of **36** as a colorless oil (117 mg, 77%). $R_f$ (EtOAc/hexanes, 8:2) = 0.22; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{68}H_{89}N_6O_9S_2^+$: 1197.6127; found: 1197.6134; *m*/*z* $[M+Na]^+$ calcd for $C_{68}H_{88}N_6O_9S_2Na^+$: 1219.5946; found: 1219.6000.

4.1.26. General procedure C: Global deprotection and cyclization

The linear precursor was dissolved in a mixture of TFA, thioanisole and $H_2O$ (90:5:5, 100 mL per mmol) and the resulting mixture was stirred at room temperature for 2-4 h. The TFA was removed under reduced

pressure and the crude product was precipitated by addition of cold diethyl ether. The diethyl ether was removed under reduced pressure and the residue was dried under high vacuum to give the crude product as a white solid.

4.1.27. 1-(3-((2*S*,6*S*,9a*S*)-2-(2-Guanidinoethyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**44a**)

Linear precursor **32** (174 mg, 0.15 mmol) was deprotected and cyclized following general procedure C. The crude product (approx. 200 mg) was dissolved in dry DMF (5 mL) and 1*H*-pyrazole-1-carboxamidine hydrochloride (305 mg, 2.06 mmol) and DIPEA (0.58 mL, 3.33 mmol) were added and the mixture was stirred under inert atmosphere for 70 h during which the reaction was monitored by RP-HPLC. Diethyl ether (20 mL) was then added and the mixture was cooled at 4 °C and stirred for an additional hour, resulting in the precipitation of a white solid. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (24 mg, 42%). $^{1}$H NMR (600 MHz, $CD_3OD$): $\delta$ = 7.86 – 7.83 (m, 2H), 7.80 (d, $J$ = 8.3 Hz, 1H), 7.64 (s, 1H), 7.50 – 7.44 (m, 2H), 7.42 (dd, $J$ = 8.5, 1.5 Hz, 1H), 5.07 (dd, $J$ = 10.0, 5.4 Hz, 1H), 5.01 (dd, $J$ = 8.8, 4.2 Hz, 1H), 3.72 – 3.69 (m, 2H), 3.43 (dd, $J$ = 12.8, 4.3 Hz, 1H), 3.29 – 3.25 (m, 1H), 3.22 – 3.16 (m, 1H), 3.15 – 3.03 (m, 5H), 2.86 – 2.80 (m, 1H), 2.59 (dd, $J$ = 16.4, 3.6 Hz, 1H), 2.49 (dd, $J$ = 16.4, 11.9 Hz, 1H), 1.87 – 1.72 (m, 3H), 1.70 – 1.64 (m, 1H), 1.52 – 1.44 (m, 2H); $^{13}$C NMR (150.9 MHz, $CD_3OD$): $\delta$ = 170.8, 169.2, 158.6, 158.5, 137.5, 135.0, 133.8, 129.4, 128.7, 128.64, 128.59, 128.5, 127.3, 126.8, 56.6, 53.1, 52.1, 49.7, 41.7, 41.6, 39.8, 35.3, 35.2, 34.1, 29.3 26.2; HRMS (ESI): $m/z$ $[M+H]^+$ calcd for $C_{26}H_{37}N_8O_2S^+$: 525.2755; found: 525.2766. Purity = 99.8% (UV 220 nm).

4.1.28. 1-(3-((2*R*,6*S*,9a*R*)-2-(2-guanidinoethyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**44b**)

Bicycle **44b** was prepared as described for **44a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (22 mg, 38%). $^{1}$H NMR (600 MHz, $CD_3OD$): $\delta$ = 7.84 – 7.78 (m 3H), 7.71 (s, 1H), 7.48 – 7.42 (m, 3H), 5.38 (dd, $J$ = 4.0, 2.8 Hz, 1H), 4.65 (app t, $J$ = 7.8 Hz, 1H), 4.20 (dt, $J$ = 13.4, 7.7 Hz, 1H), 3.98 (dd, $J$ = 15.0, 4.4 Hz, 1H), 3.69 – 3.65 (m, 1H), 3.54 (dd, $J$ = 15.0, 2.5 Hz, 1H), 3.49 – 3.44 (m ,1H), 3.17 (app t, $J$ = 7.0 Hz, 2H), 3.13 – 3.04 (m, 3H), 2.99 – 2.90 (m, 2H), 2.61 (dd, $J$ = 14.8, 3.9 Hz, 1H), 1.84 – 1.77 (m, 1H), 1.60 – 1.45 (m, 3H), 1.35 – 1.23 (m, 2H); $^{13}$C NMR (150.9 MHz, $CD_3OD$); $\delta$ = 172.3, 169.4, 158.6, 158.4, 137.2, 135.0, 133.9, 129.3, 128.7, 128.54, 128.45 (2C),

127.2, 126.7, 56.1, 55.1, 49.2 (HSQC), 48.5 (HSQC), 41.4, 40.02, 39.98, 38.1, 37.8, 34.6, 30.6, 26.1; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{26}H_{37}N_8O_2S^+$: 525.2755; found: 525.2764. Purity = 97.4 % (UV 220 nm).

4.1.29. 1,1'-(((2*S*,6*S*,9a*S*)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazine-2,6-diyl)bis(propane-3,1-diyl))diguanidine (**45a**)

Linear precursor **39** (128 mg, 111 mmol) was deprotected and cyclized following general procedure C. The crude product (58 mg) was dissolved in anhydrous DMF (4 mL) and 1*H*-pyrazole-1-carboxamidine hydrochloride (27 mg, 0.18 mmol) and DIPEA (30 µL, 0.17 mmol) were added. The reaction mixture was stirred under inert atmosphere for 144 h during which the reaction was monitored by analytical RP-HPLC. Additional 1*H*-pyrazole-1-carboxamidine hydrochloride and DIPEA was added during this time. Diethyl ether (25 mL) was added and the mixture was cooled to 0 °C and stirred for an additional hour, resulting in the precipitation of a yellow solid (203 mg). The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (6.5 mg, 15%). $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 7.86 – 7.82 (m, 2H), 7.81 – 7.77 (m, 1H), 7.62 (s, 1H), 7.51 – 7.44 (m, 2H), 7.44 – 7.40 (m, 1H), 5.15 (dd, *J* = 10.1, 5.1 Hz, 1H), 4.89 (HSQC, 1H), 3.88 – 3.81 (m, 1H), 3.58 – 3.50 (m, 1H), 3.31 (HSQC, 1H), 3.22 – 3.08 (m, 5H), 3.07 – 2.99 (m, 2H), 2.53 – 2.44 (m, 2H), 2.41 – 2.33 (m, 1H), 1.94 – 1.86 (m, 1H), 1.81 – 1.73 (m, 1H), 1.57 – 1.27 (m, 6H); $^{13}$C NMR (150.9 MHz, $CD_3OD$): $\delta$ = 170.7, 169.2, 158.62, 158.57, 137.7, 135.0, 133.8, 129.4, 128.71, 128.68, 128.67, 128.63, 127.4, 126.8, 56.6, 54.1, 51.7, 49.9, 42.2, 42.0, 41.6, 37.0, 34.1, 33.0, 29.1, 27.2, 26.2; HRMS (ESI): *m*/*z* $[M+2H]^{2+}$ calcd for $C_{27}H_{40}N_8O_2S^{2+}$: 270.1492; found 270.1499. Purity = 100.0% (UV 220 nm).

4.1.30. 1,1'-(((2*R*,6*S*,9a*R*)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazine-2,6-diyl)bis(propane-3,1-diyl))diguanidine (**45b**)

Bicycle **45b** was prepared as described for **45a**. The crude product was purified by semi-preparative RP-HPLC gave the title compound as a fluffy white solid (6.6 mg, 16%). $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 7.83 – 7.78 (m, 3H), 7.71 (s, 1H), 7.49 – 7.42 (m, 3H), 5.35 – 5.33 (m, 1H), 4.66 (t, *J* = 7.8 Hz, 1H), 4.20 – 4.14 (m, 1H), 3.95 (dd, *J* = 14.8, 4.3 Hz, 1H), 3.64 – 3.58 (m, 1H), 3.53 (dd, *J* = 14.9, 2.8 Hz, 1H), 3.51 – 3.46 (m, 1H), 3.13 – 2.92 (m, 7H), 2.59 (dd, *J* = 14.9, 3.8 Hz, 1H), 1.62 – 1.47 (m, 5H), 1.40 – 1.28 (m, 3H); $^{13}$C NMR (150.9 MHz, $CD_3OD$): $\delta$ = 172.6, 169.5, 158.6, 158.5, 137.2, 135.0, 133.9, 129.3, 128.7, 128.51, 128.45, 128.4, 127.2, 126.7, 56.1, 55.0, 49.2 (HSQC), 48.8 (HSQC), 42.0, 41.5, 40.4, 40.2, 36.2, 34.6, 30.6,

27.5, 26.2; HRMS (ESI): *m*/*z* $[M + 2H]^{2+}$ calcd for $C_{27}H_{40}N_8O_2S^{2+}$: 270.1492; found 270.1498. Purity = 95.2 % (UV 220 nm).

4.1.31. 1-(3-((2*S*,6*S*,9a*S*)-2-(4-guanidinobutyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**46a**)

Linear precursor **40** (47 mg, 0.04 mmol) was deprotected and cyclized following general procedure C. The crude product (29 mg) was dissolved in anhydrous DMF (2 mL) and 1*H*-pyrazole-1-carboxamidine hydrochloride (85 mg, 0.58 mmol) and DIPEA (0.1 mL, 0.57 mmol) were added. The reaction mixture was stirred under inert atmosphere for 94 h during which the reaction was monitored by analytical RP-HPLC. Cold diethyl ether (10 mL) was then added and the mixture was cooled at 0 °C and stirred for an additional hour, resulting in the precipitation of a white solid. The crude product was purified by semi-preparative RP-HPLC to give the title compound as fluffy white solid (3.3 mg, 22%). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.84 (t, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.61 (s, 1H), 7.50 – 7.45 (m, 2H), 7.41 (dd, *J* = 8.3, 1.8 Hz, 1H), 5.16 (dd, *J* = 10.4, 5.0 Hz, 1H), 4.88 (HSQC, 1H), 3.88 – 3.84 (m, 1H), 3.52 – 3.47 (m, 1H), 3.27 (dd, *J* = 12.6, 4.3 Hz, 1H), 3.21 – 3.11 (m, 5H), 3.06 – 3.02 (m, 1H), 2.98 (dd, *J* = 12.6, 9.9 Hz, 1H), 2.48 (dd, *J* = 16.3, 3.1 Hz, 1H), 2.45 – 2.41 (m, 1H), 2.35 (dd, *J* = 16.3, 11.9 Hz, 1H), 1.93 – 1.88 (m, 1H), 1.81 – 1.75 (m, 1H), 1.56 – 1.45 (m, 4H), 1.44 – 1.38 (m, 1H), 1.37 – 1.28 (m, 2H), 1.20 – 1.14 (m, 1H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 170.8, 169.3, 158.7, 158.6, 137.7, 135.0, 133.8, 129.4, 128.70, 128.69 (2C), 128.63, 127.4, 126.8, 56.6, 54.3, 51.6, 50.0, 42.3, 42.2, 41.6, 37.0, 35.5, 34.0, 29.5, 29.0, 26.2, 24.8; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{41}N_8O_2S$: 553.3068; found: 553.3076. Purity = 99.7% (UV 220 nm).

4.1.32. 1-(3-((2*R*,6*S*,9a*R*)-2-(4-guanidinobutyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**46b)**

Bicycles **46b** was prepared as described for **46a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compounds as a fluffy white solid (3.6 mg, 2:8 mixture between **46c**+**d** and **46b**). $^1$H NMR (850 MHz, $CD_3OD$): 7.83 – 7.78 (m, 3H), 7.71 (s, 1H), 7.48 – 7.42 (m, 3H), 5.32 (t, *J* = 3.6 Hz, 1H), 4.67 (t, *J* = 7.8 Hz, 1H), 4.16 (dt, *J* = 13.7, 7.7 Hz, 1H), 3.93 (dd, *J* = 14.8, 4.3 Hz, 1H), 3.60 – 3.56 (m, 1H), 3.53 – 3.45 (m, 2H), 3.14 – 3.04 (m, 4H), 3.00 – 2.93 (m, 3H), 2.58 (dd, *J* = 14.9, 3.9 Hz, 1H), 1.68 – 1.48 (m, 5H), 1.42 – 1.29 (m, 5H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 172.7, 169.6, 158.6, 158.5, 137.2, 135.0, 133.9, 129.3, 128.7, 128.51, 128.46, 128.43, 127.2, 126.7, 56.1, 54.9, 49.0 (2C, HSQC), 42.2, 41.5,

40.6, 40.3, 38.7, 34.6, 30.6, 29.5, 26.2, 25.1; HRMS (ESI) *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{41}N_8O_2S^+$: 553.3068; found: 553.3075.

Due to signal overlap, all chemical shifts could not be unambiguously identified for **46c**. See supporting information for key chemical shifts for **46c**.

4.1.33. 1-(3-((2*S*,6*S*,9a*S*)-2-(5-guanidinopentyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**47a**)

Linear precursor **41** (80 mg, 0.07 mmol) was deprotected and cyclized following general procedure C. The crude product (108 mg) was dissolved in anhydrous DMF (5 mL) and 1*H*-pyrazole-1-carboxamidine hydrochloride (118 mg, 0.80 mmol) and DIPEA (0.11 mL, 0.63 mmol) were added. The reaction mixture was stirred under inert atmosphere for 99 h during which the reaction was monitored by analytical RP-HPLC. Cold diethyl ether (20 mL) was then added and the mixture was cooled at 4 °C and stirred for an additional hour, resulting in the precipitation of a white solid. The crude product was purified by semi-preparative RP-HPLC to give the title compound as fluffy white solid (3.5 mg, 13%). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.84 (t, *J* = 7.7 Hz, 2H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.62 (s, 1H), 7.50 – 7.45 (m, 2H), 7.41 (dd, *J* = 8.4, 1.7 Hz, 1H), 5.16 (dd, *J* = 10.3, 5.1 Hz, 1H), 4.86 (HSQC, 1H), 3.89 – 3.85 (m, 1H), 3.51 – 3.47 (m, 1H), 3.27 (dd, *J* = 12.6, 4.4 Hz, 1H), 3.21 – 3.11 (m, 5H), 3.05 – 3.02 (m, 1H), 2.97 (dd, *J* = 12.6, 10.0 Hz, 1H), 2.48 (dd, *J* = 16.4, 3.1 Hz, 1H), 2.43 – 2.38 (m, 1H), 2.33 (dd, *J* = 16.3, 11.9 Hz, 1H), 1.94 – 1.88 (m, 1H), 1.81 – 1.75 (m, 1H), 1.59 – 1.48 (m, 4H), 1.41 – 1.36 (m, 1H), 1.34 – 1.25 (m, 4H), 1.17 – 1.10 (m, 1H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 170.8, 169.3, 158.7, 158.6, 137.7, 135.0, 133.8, 129.4, 128.71, 128.692, 128.688, 128.64 , 127.4, 126.8, 56.6, 54.4, 51.6, 50.0, 42.4, 42.3, 41.6, 37.1, 35.8, 34.0, 29.7, 29.0, 27.37, 27.36, 26.2; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{43}N_8O_2S^+$: 567.3224; found: 567.3226. Purity = 99.9% (UV 220 nm).

4.1.34. 1-(3-((2*R*,6*S*,9a*R*)-2-(5-guanidinopentyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**47b)**

Bicycles **47b** and was prepared as described for **47a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compounds as a fluffy white solid (3.5 mg, 13%). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.84 – 7.78 (m, 3H), 7.71 (s, 1H), 7.48 – 7.42 (m, 3H), 5.31 (t, *J* = 3.7 Hz, 1H), 4.67 (t, *J* = 7.8 Hz, 1H), 4.18 – 4.13 (m, 1H), 3.92 (dd, *J* = 14.8, 4.3 Hz, 1H), 3.59 – 3.55 (m, 1H), 3.52 – 3.48 (m, 2H), 3.17 – 3.11 (m, 2H), 3.11 – 3.05 (m, 2H), 3.01 – 2.94 (m, 3H), 2.57 (dd, *J* = 14.9, 4.0 Hz, 1H), 1.61 – 1.49 (m, 5H), 1.39 – 1.29 (m, 7H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 172.7, 169.5, 158.6, 158.5, 137.2, 135.0,

133.9, 129.3, 128.7, 128.51, 128.46, 128.43, 127.2, 126.7, 56.0, 54.8, 49.2 (HSQC), 49.1 (HSQC), 42.3, 41.5, 40.6, 40.4, 39.1, 34.6, 30.6, 29.7, 27.6, 27.3, 26.2; HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{43}N_8O_2S^+$: 567.3224; found: 567.3225. Purity = 98.9% (UV 220 nm).

4.1.35. 1,1'-(((2*S*,6*S*,9a*S*)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazine-2,6-diyl)bis(propane-3,1-diyl))diguanidine (**48a**)

Linear precursor **42** (90 mg, 0.08 mmol) was deprotected and cyclized following general procedure C. The crude product (97 mg) was dissolved in anhydrous DMF (2 mL) and 1*H*-pyrazole-1-carboxamidine hydrochloride (85 mg, 0.58 mmol) and DIPEA (0.10 mL, 0.57 mmol) were added. The reaction mixture was stirred under inert atmosphere for 78 h during which the reaction was monitored by analytical RP-HPLC. Cold diethyl ether (10 mL) was then added and the mixture was cooled at 4 °C and stirred for an additional hour, resulting in the precipitation of a white solid. The crude product was purified by semi-preparative RP-HPLC to give the title compound as fluffy white solid (3.3 mg, 11%). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.82 – 7.78 (m, 2H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.69 (s, 1H), 7.46 – 7.41 (m, 2H), 7.37 (dd, *J* = 8.4, 1.8 Hz, 1H), 5.01 (dd, *J* = 10.1, 5.0 Hz, 1H), 4.95 (dd, *J* = 8.4, 4.7 Hz, 1H), 3.68 – 3.63 (m, 1H), 3.54 – 3.48 (m, 2H), 3.41 – 3.37 (m, 1H), 3.27 – 3.23 (m, 1H), 3.22 – 3.16 (m, 3H), 3.02 – 2.98 (m, 1H), 2.90 – 2.86 (m, 1H), 2.84 – 2.80 (m, 1H), 2.58 (dd, *J* = 16.6, 3.4 Hz, 1H), 2.42 (dd, *J* = 16.5, 12.0 Hz, 1H), 2.11 – 2.03 (m, 2H), 1.97 – 1.92 (m, 1H), 1.80 – 1.74 (m, 1H), 1.71 – 1.65 (m, 1H), 1.64 – 1.57 (m, 4H), 1.52 – 1.47 (m, 1H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 170.9, 169.2, 158.7, 158.6, 140.5, 135.1, 133.6, 129.1, 128.7, 128.5, 128.1, 127.4, 127.1, 126.4, 56.7, 52.6, 52.3, 48.3, 42.1, 42.0, 41.7, 37.1, 34.5, 33.3, 29.7, 28.9, 27.2, 26.4; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{41}N_8O_2S^+$: 553.3068; found: 553.3077. Purity = 100.0% (UV 220 nm).

4.1.36. 1,1'-(((2*R*,6*S*,9a*R*)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazine-2,6-diyl)bis(propane-3,1-diyl))diguanidine (**48b**)

Bicycle **48b** was prepared as described for **48a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (4.6 mg, 2:8 mixture between **48c** and **48b**). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.82 – 7.77 (m, 3H), 7.68 (s, 1H), 7.46 – 7.40 (m, 2H), 7.40 – 7.37 (m, 1H), 5.41 – 5.40 (m, 1H), 4.79 – 4.76 (m, 1H), 4.11 (dd, *J* = 14.9, 4.3 Hz, 1H), 3.67 – 3.63 (m, 1H), 3.59 – 3.55 (m, 1H), 3.53 (dd, *J* = 15.0, 2.7 Hz, 1H), 3.52 – 3.48 (m, 1H), 3.27 – 3.18 (m, 3H), 3.12 – 3.09 (m, 2H), 3.05 (dd, *J* = 14.8, 6.0 Hz, 1H), 2.91 – 2.86 (m, 1H), 2.83 – 2.79 (m, 1H), 2.63 (dd, *J* = 14.9, 3.8 Hz, 1H), 2.09 – 1.97 (m, 2H), 1.83 – 1.75 (m, 2H), 1.69 – 1.65 (m, 2H), 1.64 – 1.57 (m, 3H), 1.46 – 1.39 (m, 1H); $^{13}$C NMR

(850 MHz, $CD_3OD$): $\delta$ = 172.7, 169.9, 158.61, 158.55, 140.3, 135.2, 133.6, 129.1, 128.6, 128.4, 128.1, 127.4, 127.0, 126.3, 56.1, 55.1, 48.4, 47.8, 42.0, 41.7, 40.4, 40.3, 36.3, 34.3, 30.7, 29.8, 27.5, 26.5; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{41}N_8O_2S^+$: 553.3068; found: 553.3077. Purity = 97.2% (sum of diastereoisomers **48b** and **48c**, UV 220 nm)

Due to signal overlap, all chemical shifts could not be unambiguously identified for **48c**. See supporting information for key chemical shifts for **48c**.

4.1.37. 1,1'-(((2*RS*,6*S*,9a*RS*)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazine-2,6-diyl)bis(propane-3,1-diyl))diguanidine (**48d)**

Bicycle **48d** was prepared as described for **48a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (0.9 mg, 2:8 mixture of **48b** and **48d**). $^1H$ (850 MHz, $CD_3OD$): $\delta$ = 7.82 – 7.77 (m, 3H), 7.68 (s, 1H), 7.45 – 7.41 (m, 2H), 7.38 (dd, *J* = 8.3, 1.7 Hz, 1H), 4.66 (dd, *J* = 9.2, 6.0 Hz, 1H), 4.50 (dd, *J* = 11.4, 4.4 Hz, 1H), 3.72 – 3.68 (m, 1H), 3.67 – 3.63 (m, 1H), 3.59 (dd, *J* = 13.7, 4.3 Hz, 1H), 3.49 – 3.44 (m, 1H), 3.26 – 3.19 (m, 3H), 3.16 (t, *J* = 6.8 Hz, 2H), 2.91 – 2.87 (m, 1H), 2.83 – 2.79 (m, 1H), 2.62 (dd, *J* = 16.1, 5.3 Hz, 1H), 2.43 (dd, *J* = 16.2, 7.1 Hz, 1H), 2.08 – 2.00 (m, 2H), 1.85 – 1.80 (m, 1H), 1.78 – 1.73 (m, 1H), 1.70 – 1.65 (m, 3H), 1.65 – 1.59 (m, 3H), 1.51 – 1.46 (m, 1H); $^{13}C$ (213.8 MHz, $CD_3OD$): $\delta$ = 171.3, 170.2, 158.64, 158.59, 140.6, 135.1, 133.6, 129.0, 128.6, 128.5, 128.3, 127.4, 127.2, 126.5, 57.4, 56.5, 48.2, 47.8, 41.9, 41.6, 41.0, 40.8, 35.1, 34.5, 31.2, 29.6, 27.3, 26.5; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{41}N_8O_2S^+$: 553.3068; found: 553.3076. Purity = 82.7% (sum of diastereoisomers **48b** and **48d**, UV 220 nm)

4.1.38. 1-(3-((2*S*,6*S*,9a*S*)-2-(5-guanidinopentyl)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**49a**)

Linear precursor **43** (117 mg, 0.10 mmol) was deprotected and cyclized following general procedure C. The crude product (87 mg) was dissolved in anhydrous DMF (5 mL), 1*H*-pyrazole-1-carboxamidine hydrochloride (100 mg, 0.69 mmol) and DIPEA (0.12 mL, 0.69 mmol) were added. The reaction mixture was stirred at room temperature for 93 h at room temperature during which the reaction was monitored by analytical RP HPLC. The reaction was cooled to 0 °C, cold diethyl ether (20 mL) was added and stirring continued for an additional hour, resulting in the precipitation of a brown oil (152 mg). The crude product was purified by semi-preparative RP-HPLC to give the title compound as a white fluffy solid (3.2 mg, 8%). $^1H$ NMR (850 MHz, $CD_3OD$): $\delta$ = 7.80 (dd, *J* = 17.7, 8.0 Hz, 2H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.69 (s, 1H),

7.46 – 7.41 (m, 2H), 7.36 (dd, *J* = 8.4, 1.7 Hz, 1H), 5.01 (dd, *J* = 10.1, 4.9 Hz, 1H), 4.91 (dd, *J* = 8.8, 4.5 Hz, 1H), 3.67 (dt, *J* = 13.6, 7.4 Hz, 1H), 3.52 – 3.45 (m, 2H), 3.37 (ddd, *J* = 13.4, 7.4, 6.0 Hz, 1H), 3.27 – 3.23 (m, 1H), 3.21 – 3.15 (m, 3H), 2.97 – 2.93 (m, 1H), 2.90 – 2.86 (m, 1H), 2.84 – 2.79 (m, 1H), 2.55 (dd, *J* = 16.6, 3.4 Hz, 1H), 2.39 (dd, *J* = 16.6, 12.0 Hz, 1H), 2.11 – 2.03 (m, 2H), 1.97 – 1.92 (m, 1H), 1.80 – 1.74 (m, 1H), 1.63 – 1.57 (m, 4H), 1.57 – 1.53 (m, 1H), 1.48 – 1.41 (m, 2H), 1.41 – 1.34 (m, 3H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 171.0, 169.3, 158.65, 158.60, 140.5, 135.1, 133.5, 129.1, 128.7, 128.5, 128.1, 127.4, 127.1, 126.3, 56.7, 52.8, 52.1, 48.3, 42.4, 42.2, 41.7, 37.3, 36.2, 34.5, 29.73, 29.67, 28.9, 27.43, 27.38, 26.3; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{45}N_8O_2S^+$: 581.3381; found: 581.3389. Purity = 99.0% (UV 220 nm).

4.1.39. 1-(3-((2*R*,6*S*,9a*R*)-2-(5-guanidinopentyl)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**49b)**

Bicycles **49b** was prepared as described for **49a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (2.2 mg, 3:7 mixture between **49c** and **49b**). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.81 – 7.77 (m, 3H), 7.69 (s, 1H), 7.46 – 7.37 (m, 3H), 5.40 – 5.39 (m, 1H), 4.80 – 4.77 (m, 1H), 4.10 (dd, *J* = 14.9, 4.3 Hz, 1H), 3.67 – 3.63 (m, 1H), 3.63 – 3.59 (m, 1H), 3.53 (dd, *J* = 15.0, 2.7 Hz, 1H), 3.47 – 3.43 (m, 1H), 3.27 – 3.20 (m, 2H), 3.17 (t, *J* = 7.2 Hz, 1H), 3.05 – 3.01 (m, 2H), 2.92 – 2.87 (m, 1H), 2.83 – 2.78 (m, 1H), 2.62 (dd, *J* = 14.9, 3.8 Hz, 1H), 2.10 – 2.02 (m, 1H), 2.01 – 1.96 (m, 1H), 1.84 – 1.75 (m, 2H), 1.70 – 1.64 (m, 2H), 1.62 – 1.56 (m, 2H), 1.47 – 1.42 (m, 1H), 1.41 – 1.24 (m, 5H); $^{13}$C (213.8 MHz, $CD_3OD$): $\delta$ = 172.9, 169.9, 158.6, 158.5, 140.4, 135.2, 133.6, 129.0, 128.6, 128.4, 128.1, 127.4, 127.0, 126.3, 56.1, 55.0, 48.4, 47.7, 42.3, 41.7, 40.8, 40.5, 39.3, 34.3, 30.7, 29.9, 29.6, 27.7, 27.3, 26.5; HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{45}N_8O_2S^+$: 581.3381; found: 581.3382; Purity = 96.6% (sum of diastereoisomers **49b** and **49c**, UV 220 nm).

Due to signal overlap, all chemical shifts could not be unambiguously identified for **49c**. See supporting information for key chemical shifts for **49c**.

4.1.40. 1-(3-(2-(5-guanidinopentyl)-8-(3-(naphthalen-2-yl)propyl)-4,7-dioxohexahydro-2*H*,6*H*-pyrazino[2,1-*b*][1,3]thiazin-6-yl)propyl)guanidine (**49d**)

Bicycle **49d** was prepared as described for **49a** above. The crude product was purified by semi-preparative RP-HPLC to give the title compound as a fluffy white solid (1.3 mg, 1:9 mixture of **49b** and **49d**). $^1$H NMR (850 MHz, $CD_3OD$): $\delta$ = 7.82 – 7.77 (m, 3H), 7.68 (s, 1H), 7.46 – 7.41 (m, 2H), 7.39 (dd, *J* = 8.3, 1.8 Hz,

1H), 4.65 (dd, *J* = 9.0, 6.1 Hz, 1H), 4.49 (dd, *J* = 11.4, 4.5 Hz, 1H), 3.72 – 3.68 (m, 1H), 3.66 (dd, *J* = 13.6, 11.4 Hz, 1H), 3.58 (dd, *J* = 13.7, 4.4 Hz, 1H), 3.49 – 3.45 (m, 1H), 3.26 – 3.19 (m, 3H), 3.16 (t, *J* = 7.2 Hz, 2H), 2.91 – 2.87 (m, 1H), 2.83 – 2.79 (m, 1H), 2.59 (dd, *J* = 16.0, 5.5 Hz, 1H), 2.41 (dd, *J* = 16.1, 6.9 Hz, 1H), 2.09 – 1.99 (m, 2H), 1.85 – 1.80 (m, 1H), 1.78 – 1.72 (m, 1H), 1.70 – 1.65 (m, 2H), 1.61 – 1.56 (m, 3H), 1.48 – 1.34 (m, 5H); $^{13}$C NMR (213.8 MHz, $CD_3OD$): $\delta$ = 171.6, 170.2, 158.63, 158.59, 140.6, 135.1, 133.6, 129.0, 128.6, 128.5, 128.3, 127.4, 127.2, 126.5, 57.4, 56.4, 48.2, 47.8, 42.4, 41.6, 41.1, 41.0, 38.2, 34.5, 31.2, 29.7, 29.6, 27.5, 27.3, 26.5: HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{30}H_{45}N_8O_2S^+$: 581.3381; found 581.3389. Purity = 90.9% (UV 220 nm).

**4.2. Biology** – The final compounds were tested for antagonistic activity against the human CXCR4 in a scintillation proximity-based inositol-phosphate accumulation assay (SPA-IP) following the same protocol as recently described.[7]

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**Supplementary Data**

Supplementary data (HPLC traces for key cyclization reactions and key NMR data for bicycles) associated with this article can be found, in the online version, at http://

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