Design, Synthesis, and Biological Evaluation of Scaffold-Based Tripeptidomimetic Antagonists for CXC Chemokine Receptor 4 (CXCR4)

Zack G. Zachariassen, a Stefanie Thiele, b Erik A. Berg, c Pernille Rasmussen, c Torgils Fossen, c Mette M. Rosenkilde, b Jon Våbenø,* a Bengt Erik Haug* c

a Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Breivika, NO-9037 Tromsø, Norway.
b Laboratory for Molecular Pharmacology, Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark.
c Department of Chemistry and Centre for Pharmacy, University of Bergen, Allégaten 41, NO-5007 Bergen, Norway.

Corresponding author for the review process:
Bengt Erik Haug. Phone: +47 55 58 34 68; e-mail: Bengt-Erik.Haug@farm.uib.no

Post-publication corresponding authors:
* Bengt Erik Haug. Phone: +47 55 58 34 68; e-mail: Bengt-Erik.Haug@farm.uib.no
or
* Jon Våbenø. Phone: +47 77 62 09 09; e-mail: jon.vabeno@uit.no

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Abstract
Structure-activity relationship studies of the cyclopentapeptide CXCR4 antagonists (cyclo(-L-/D-Arg\textsuperscript{1}-Arg\textsuperscript{2}-2-Nal\textsuperscript{3}-Gly\textsuperscript{4}-D-Tyr\textsuperscript{5}-)) suggest that the L-/D-Arg\textsuperscript{1}-Arg\textsuperscript{2}-2-Nal\textsuperscript{3} tripeptide sequence contained within these cyclopentapeptides serves as a recognition motif for peptidic CXCR4 antagonists. Starting by dissecting the cyclopentapeptide structure and reintroducing cyclic constraints in a stepwise manner, we here report a novel class of scaffold-based tripeptidomimetic CXCR4 antagonists based on the D-Arg-Arg-2-Nal motif. Biological testing of the prototype compounds showed that they represent new peptidomimetic hits; importantly, the modular nature of the scaffold provides an interesting starting point for future ligand optimization.

Graphical Abstract
1. INTRODUCTION

CXC chemokine receptor 4 (CXCR4) is a peptidergic GPCR with the 68-residue peptide CXC chemokine ligand 12 (CXCL12) as its only endogenous ligand.\(^1,2\) In addition to the developmental and physiological role of CXCL12/CXCR4, CXCR4 has been shown to be involved in a number of pathological conditions, including HIV, cancer, and rheumatoid arthritis.\(^3\) Consequently, CXCR4 has emerged as an attractive drug target, and several small-molecule CXCR4 antagonists have been described in the literature over the last decade,\(^3,4\) including a series of cyclic pentapeptides based on the amino acid sequence L-/D-Arg\(^1\)-Arg\(^2\)-2-Nal\(^3\)-Gly\(^4\)-D-Tyr\(^5\) (2-Nal = L-3-(2-naphthyl)alanine), i.e. the L-Arg\(^1\) epimer 1 (FC131) and the D-Arg\(^1\) epimer 2 (FC092) (Figure 1).\(^5\)

![Figure 1. Structures of the lead cyclopentapeptide antagonists 1 and 2.](image)

Small cyclic peptides, such as cyclopentapeptides, are known to mimic peptide turns,\(^6\) i.e. structural motifs where the peptide backbone folds back on itself to form a pseudo-cyclic structure. Such turns appear to be a universal ligand recognition element for peptidergic GPCRs;\(^7\) thus, the cyclopentapeptide CXCR4 antagonists represent an excellent starting point for design of novel CXCR4 antagonists based on smaller turn-mimicking scaffolds. Such scaffolds have to maintain the 3D-orientation of the pharmacophoric groups of the parent peptide, resulting in a so-called topographical (or type-III) peptidomimetic.\(^8\) With respect to size, a recent review points out that three amino acids represent an optimal size for small-molecule peptidomimetics.\(^9\)

The Gly\(^4\) residue in the cyclopentapeptide CXCR4 antagonists (Figure 1) was originally introduced for synthetic reasons\(^5\) and thus can be considered as a spacer. Through SAR studies, it has become clear that the D-Tyr\(^5\) side chain is not essential for biological activity,\(^10,11\) and we have in a recent molecular docking study found that this may stem from the lack of a defined binding pocket for the D-Tyr\(^5\) side chain.\(^11\) Even if our modeling
suggested that D-Tyr$^5$ of 1 is partly solvent exposed, the phenolic hydroxyl group was found to take part in hydrogen bonding to Glu$^{32,11}$ However, as the D-Phe$^5$ analogue was found to be only 2-fold less potent, this H-bond is not essential for antagonistic activity; indeed, the Gly$^5$ analogue of 1 was found to be only one order of magnitude less potent than 1 in our assay system.$^{11}$ Collectively, these observations imply that the remaining L-/D-Arg$^1$-Arg$^2$-2-Nal$^3$ tripeptide fragment serves as a recognition motif for peptidic CXCR4 antagonists, which motivates further studies of both flexible and constrained small molecules containing this motif. Through dissection of the cyclopentapeptide structure and a stepwise reintroduction of cyclic constraints, we here report the design, synthesis, and biological evaluation of a novel class of scaffold-based tripeptidomimetic CXCR4 antagonists based on the D-Arg-Arg-2-Nal motif.

2. RESULTS AND DISCUSSION

2.1. General Design Considerations.

As SAR studies of the cyclopentapeptide CXCR4 antagonists (Figure 1) have demonstrated that position 2 (L-Arg) is very sensitive to structural modifications,$^{12,13}$ we decided to keep L-Arg$^2$ throughout this study. Similarly, we have recently shown that replacement of L-2-Nal in position 3 with aromatic/alicyclic analogs results in significant reduction of the antagonistic potency,$^{11}$ and therefore used a 2-naphthyl group with the appropriate spacer length for all compounds. In contrast, position 1 has been shown to be relatively tolerant to structural modifications, both with respect to stereochemistry (L- or D-arginine) and the chemical nature of the side chain.$^{13,14}$ Even if the originally discovered L-Arg$^1$ epimer 1 displays somewhat higher affinity than the D-Arg$^1$ epimer 2 (Figure 1; IC$_{50}$-values of 0.004 and 0.008 µM, respectively),$^5$ subsequent SAR studies have shown that cyclopentapeptide analogs containing D-Arg$^1$ in many cases are more active than the corresponding L-Arg$^1$ epimers. For example, the most active cyclopentapeptide CXCR4 antagonist reported to date is the N-methylated D-Arg$^1$ epimer FC122 (cyclo(-N-Me-D-Arg$^1$-Arg$^2$-2-Nal$^3$-Gly$^4$-D-Tyr$^5$-)), which showed 8-fold higher affinity than the corresponding L-Arg$^1$ epimer (IC$_{50}$-values of 0.003 and 0.023 µM, respectively).$^{10}$ Moreover, head-to-tail cyclization of peptides is known to be facilitated by incorporation of a D-amino acid in an all-L sequence due to a turn-inducing effect.$^{15}$ For these reasons, we decided to focus on the D-Arg$^1$ epimers in the present study, using the lead cyclopentapeptide 2 (cyclo(-D-Arg$^1$-Arg$^2$-2-Nal$^3$-Gly$^4$-D-Tyr$^5$-), Figure 2A) as starting point.
2.2. Biological Evaluation.

The antagonistic potency of the synthesized compounds 2–14 (Figures 2 and 6) on human CXCR4 was determined by a functional assay as previously described\textsuperscript{13} and is shown in Table 1; the EC\textsubscript{50}-value of the known lead compound 2 was 0.52 \(\mu\)M.

<table>
<thead>
<tr>
<th>Compd\textsuperscript{a}</th>
<th>(\log EC_{50} \pm SEM)</th>
<th>(EC_{50}) ((\mu)M)</th>
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<tr>
<td>2\textsuperscript{c}</td>
<td>-6.28 ± 0.09</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
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</tr>
<tr>
<td>6</td>
<td>&gt; -4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7</td>
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<tr>
<td>8</td>
<td>&gt; -4</td>
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<tr>
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<td>&gt;100</td>
</tr>
<tr>
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</tr>
<tr>
<td>11</td>
<td>&gt; -4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12</td>
<td>&gt; -4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>13</td>
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<td>80</td>
</tr>
<tr>
<td>14</td>
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</table>

\textsuperscript{a}For structures, see Figures 2 and 6.

\textsuperscript{b}Values represent the mean of at least three independent experiments performed in duplicates.

\textsuperscript{c}Known compound.\textsuperscript{5}
2.3. Design and SAR.

2.3.1. Linear Tripeptidic Compounds.

Taking a minimalist approach, 2 was initially dissected to the linear peptidic derivative 3 (Figure 2B) in order to determine the activity of the isolated Arg\(^1\)-Arg\(^2\)-2-Nal\(^3\) motif. The N-acetylated d-Arg\(^1\)-Arg\(^2\)-2-Nal\(^3\) tripeptide amide 4 (Figure 2B) was also included to study the role of the two flanking amide groups. Compound 3 (EC\(_{50} = 58 \mu M\)) displayed 112-fold lower potency than 2, reflecting the extensive dissection of the cyclopentapeptide structure. This finding is consistent with literature data for similar linear tripeptidic CXCR4 antagonists originating from an Arg-Arg-Nal motif;\(^{16,17}\) representative structures are shown in Figure 3.

In the same way as 3, these compounds were based on a central L-arginine, and were found to have moderate potency (anti-HIV assay), typically in the order of 100-fold reduction relative to the parent cyclopentapeptide.
Figure 3. Structures of previously reported linear tripeptic CXCR4 antagonists.16,17

The N- and C-terminal capped linear tripeptide 4 showed somewhat lower activity (EC\textsubscript{50} = 86 \mu M) than 3; thus, the terminal amide groups of 4 do not contribute favorably to activity. The activity of 4 relative to 2 (165-fold reduction) is consistent with SAR studies by Fujii \textit{et al.} which showed that the linear N- and C-terminal capped pentapeptide Ac-D-Arg\textsuperscript{1}-L-Arg\textsuperscript{2}-L-2-Nal\textsuperscript{3}-Gly\textsuperscript{4}-D-Tyr\textsuperscript{5}-NH\textsubscript{2} was 173-fold less potent (anti-HIV assay) than the parent cyclopentapeptide 2.5

2.3.2. Macrocyclic Compounds.

The analogs 3 and 4 are quite flexible, which is generally considered as an undesirable feature, and a macrocyclic constraint was reintroduced to force the D-Arg\textsuperscript{1}-Arg\textsuperscript{2}-2-Nal\textsuperscript{3} motif into a more restricted conformation (Figure 2C). Use of a Gly\textsuperscript{4}-Gly\textsuperscript{5} dipeptide spacer to give a simplified cyclopentapeptide (5) resulted in a slight increase in potency (EC\textsubscript{50} = 44 \mu M) relative to 4. Since the Gly\textsuperscript{4}-Xaa\textsuperscript{5} amide bond was not found to be involved in direct binding interactions with the receptor in our recently proposed binding mode,\textsuperscript{11} 5 was further simplified by replacing Gly\textsuperscript{4}-Gly\textsuperscript{5} with a 5-aminopentanoic acid\textsuperscript{4} hydrocarbon spacer (6). This modification, which essentially is an ethylene amide bond isostere (Ψ[CH\textsubscript{2}CH\textsubscript{2}]) replacement, resulted in loss of activity in our assay (EC\textsubscript{50} > 100 \mu M). This shows that the planar and polar Gly\textsuperscript{4}-Gly\textsuperscript{5} amide bond in 5 contributes favorably to activity, either by geometrical or solvation effects.
Next, we employed a ring expansion/contraction strategy by using ω-amino carboxylic acid spacers of different length (7–10, Figure 2C). Extension of the hydrocarbon spacer in 6 (15-membered ring) to give a 16-membered ring (7) or contraction to give a 14-membered ring (8) did still not give any measurable activity (EC$_{50}$ > 100 µM for both compounds). Similarly, the 13-membered ring (9) was inactive (EC$_{50}$ > 100 µM); however, the further constrained cyclotetrapeptide 10 (12-membered ring) was equipotent (EC$_{50}$ = 60 µM) with the linear tripeptidic compound 3.

### 2.3.3. Bicyclic Tripeptidomimetics.

Encouraged by the activity of 10, we set out to develop a further constrained turn-mimicking scaffold capable of presenting the side chains and backbone of the D-Arg$^{1}$-Arg$^{2}$-2-Nal$^{3}$ fragment of the cyclopentapeptide 2 in the required 3D-orientation. The key to successful development of such topographical peptidomimetics is knowledge of the bioactive conformation of the parent peptide, in this case the cyclopentapeptide ligands. Based on an extensive exploration of the conformational space for a series of cyclopentapeptide CXCR4 antagonists from the literature, we have previously reported a 3D pharmacophore model that describes the spatial arrangement of the pharmacophoric side chains as well as the bioactive conformation of the cyclopentapeptide backbone.$^{18}$

Searching through the extensive literature on turn-mimetics (see ref$^{19}$ for a review), we were intrigued by the tripeptide-derived 3,6,8-trisubstituted$^{20}$ bicyclic structure A (Figure 4A),$^{21,22}$ which contains two endocyclic amide bonds. The synthesis of A was first reported by Vojkovsky et al. who suggested it as a potential peptide-turn motif;$^{21}$ however, no biological applications of A have yet been reported. In order to elucidate whether this scaffold would be suitable for our purpose, a structural comparison of low-energy conformations of A with our 3D pharmacophore model was undertaken. This showed that scaffold A is able to orient the side chains in a similar way as the parent cyclopentapeptide (Figure 4B).
**Figure 4.** (A) Structure of scaffold A, and (B) superimposition of a low-energy conformation of A (grey carbon atoms) and the bioactive backbone conformation of the cyclopentapeptide CXCR4 antagonists (green carbon atoms) as defined by our 3D pharmacophore model.\(^\text{18}\) RMSD = 0.160 Å (cf. section 4.2).

Scaffold A contains three stereocenters, where two (C3 and C6) are defined by the building blocks (see Scheme 4 in Chemistry section). The bridge-head stereocenter (C9a) is formed in the final cyclization step, and the stereochemical outcome has been shown to be governed by the configuration at C3 (see Chemistry section);\(^\text{23}\) thus, two diastereomeric scaffolds A and A’ (Figure 5A) can be prepared. Interestingly, structural comparison of the expected bioactive conformation of A (shown in Figure 4B) with low-energy conformations of A’ showed that A’ can adopt an almost identical conformation with respect to the orientation of the two amide bonds and the three side chains (Figure 5B).

**Figure 5.** (A) Structures of the diastereomeric scaffolds A and A’, and (B) superimposition of low-energy conformations of A (grey carbon atoms) and A’ (green carbon atoms). RMSD = 0.036 Å (cf. section 4.2).

Thus, we decided to pursue tripeptidomimetic compounds 11 and 12 (based on scaffold A and A’, respectively; Figure 6) by using L- and D-cysteine as building blocks, resulting in an amide group in the R\(^1\) side chain. In order to introduce the same arginine R\(^1\) side chain as the parent cyclopentapeptide, we also adapted the synthesis to allow for preparation of 13 and 14 (Figure 6), which in contrast to 11 and 12 do not carry this amide bond.
Interestingly, compounds 11 and 12 were inactive, while compounds 13 and 14 (EC$_{50}$ = 80 and 64 µM, respectively) showed activity similar to 3 and 10. Clearly, the amide bond in the R$^1$ side-chain of 11 and 12 is unfavorable for the biological activity, an observation that justifies the synthetic effort put into the R$^1$ building block 33 that was used for 13 and 14 (see Scheme 7, Chemistry section). Thus, 13 and 14 represent new peptidomimetic hit compounds for CXCR4 antagonism.

The scaffold-based tripeptidomimetics 13 and 14 are significantly less potent (154- and 123-fold, respectively) than the optimized cyclopentapeptide 2 that they are based on. This was an expected consequence of the rather extensive structural changes that were needed in order to arrive at downsized structures. To our knowledge, there are only two other examples of scaffold-based tripeptidomimetic CXCR4 antagonists in the literature (Figure 7): Niida et al. used a 1,3,6-trisubstituted 1,6-dihydropyridin-2-one scaffold, while Ueda et al. have reported a series of achiral CXCR4 antagonists based on a 1,2,5-trisubstituted indole scaffold. Also for these compounds, a significant drop in activity compared to the parent cyclopentapeptides was observed; the same group has typically reported an IC$_{50}$-value of 0.008 µM for the cyclopentapeptide 2, which means that the affinity reduction for the dihydropyridin-2-one based compound (IC$_{50}$ = 15.1 µM) and the indole-based compound (IC$_{50}$ = 3.0 µM) was 1888- and 375-fold, respectively. These numbers reflect the general complexity of the initial “scaffold jump” for prototype compounds, as also seen in the present study.
The moderate activity of our prototype bicyclic tripeptidomimetics 13 and 14 also means that binding mode studies (typically performed by combining site-directed receptor mutagenesis studies and molecular docking) are not expected to provide reliable data for these compounds. Thus, further SAR studies, aimed at optimizing both the scaffold core and the side chains, are currently in progress.

2.4. Chemistry.

The macrocyclic compounds 2 and 5–10 were prepared by head-to-tail macrolactamization of linear precursors (Scheme 1) that were obtained through Fmoc-based solid phase peptide synthesis (SPPS). The linear precursors were prepared using either a preloaded Fmoc-Gly trityl resin (2, 5, and 10) or a 2-chlorotrityl chloride resin for the loading of the N-Fmoc ω-amino carboxylic acids (6–9). Cleavage from the resin was facilitated using hexafluoroisopropanol and the side-chain protected peptides were cyclized using PyBOP followed by global deprotection using TFA.
Scheme 1. Synthesis of 2 and 5–10. Reagents and conditions: (a) N-Fmoc ω-amino-carboxylic acid, DIPEA, CH₂Cl₂, rt, 1 h; (b) MeOH, DIPEA, CH₂Cl₂, rt, 2 x 5 min; (c) HFIP, CH₂Cl₂, rt, 15 min, then 2 x 10 min; (d) PyBOP, DIPEA, DMF/CH₂Cl₂, rt, 24 h; (e) TFA/TIS/H₂O, rt, 2 h.

Compound 3 was synthesized (Scheme 2) by coupling protected arginine 15 with 2-(naphthalene-2-yl)ethanamine to give 16, which in turn was Fmoc-deprotected and coupled with N-Boc 5-aminopentanoic acid to give 17. Global deprotection facilitated by TFA followed by guanidinylation of the primary amine gave 3.

Scheme 2. Synthesis of 3. Reagents and conditions: (a) 2-(naphthalene-2-yl)ethanamine hydrochloride, HBTU, DIPEA, DMF, rt, 20 h (67%); (b) 2-ethanolamine, DMF, rt, 3 h; (c) N-Boc 5-aminopentanoic acid, HATU, DIPEA, DMF, 24 h; (d) TFA/TIS/H₂O, rt, 2 h; (e) 1H-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, rt, 48 h (21% over four steps).

The linear tripeptide 4 was prepared by Fmoc-based SPPS on an Fmoc-NH-Rink amide resin (Scheme 3) followed by acetylation of the N-terminal using acetic anhydride. Cleavage from the resin and global deprotection mediated by TFA gave the desired N-acetylated tripeptide amide 4.
The key step in the synthesis of the bicyclic tripeptidomimetics 11–14 (Scheme 4) is the spontaneous formation of the 6,6-fused bicyclic ring system A upon treatment of acetal C with TFA.\textsuperscript{21,22} The resulting aldehyde condensates with the backbone amide nitrogen to give the N-acyliminium ion intermediate B, which subsequently undergoes nucleophilic attack from the deprotected thiol, resulting in the formation of the desired bicycle A.\textsuperscript{21,22} The cyclization occurs stereoselectively,\textsuperscript{23} and the configuration at the bridge-head (C9a) is dependent on the configuration at C3 (R\textsuperscript{1} substituent), and in the absence of a R\textsuperscript{1} substituent on the configuration at C6.

**Scheme 3.** Synthesis of 4. *Reagents and conditions:* (a) Ac\textsubscript{2}O, DIPEA, DMF, rt, 30 min; (b) TFA/TIS/H\textsubscript{2}O, rt, 3 h.

Synthesis of the linear precursor C requires access to the three building blocks D, E and F. For the synthesis of target compounds 11 and 12, the R\textsuperscript{1}-side chain (incorporated through building block F) was introduced as guanidinylated glycine 20 (Scheme 5). This material was prepared by guanidinylation of glycine methyl ester hydrochloride (18) using N,N-di-Boc-1H-pyrazole-1-carboxamidine followed by hydrolysis of the methyl ester of the resulting 19 using LiOH in a mixture of water and acetone.\textsuperscript{26,27}

**Scheme 4.** Scaffold and retrosynthetic strategy.

**Scheme 5.** Synthesis of carboxylic acid 20. *Reagents and conditions:* (a) N,N-di-Boc-1H-pyrazole-1-carboxamidine, DIPEA, DMF, rt, 14 h (85%); (b) LiOH, H\textsubscript{2}O/acetone, rt, 1.5 h (99%).

Synthesis of the bicyclic core (Scheme 6) commenced with the alkylation of 2-(naphthalene-2-yl)ethanamine (21) with bromoacetaldehyde dimethyl acetal in refluxing THF to give
secondary amine 22. This amine was in turn coupled with protected arginine (15) to give 23 in high yield. Further Fmoc-deprotection and coupling with appropriately protected L-cysteine gave 24, which was submitted to another Fmoc-deprotection and then coupled with carboxylic acid 20 to give the linear precursor 25. This material was treated with TFA, thioanisole and water to facilitate global deprotection, leading to formation of the acyliminium ion intermediate that after nucleophilic attack by the thiol gave 11. The diastereomeric 12 was prepared by coupling of Fmoc-D-Cys(Trt)-OH to Fmoc-deprotected 23 to give intermediate 26 (see Experimental section), which in turn was converted to linear precursor 27 followed by deprotection and cyclization to give 12.

**Scheme 6.** Synthesis of the bicyclic tripeptidomimetic 11. Reagents and conditions: (a) BrCH₂CH(OMe)₂, THF, reflux, 24 h (30%); (b) Fmoc-Arg(Pbf)-OH (15), HATU, DIPEA, DMF, rt, 16 h (83%); (c) Et₂HN, CH₂Cl₂, rt, 2 h; (d) Fmoc-L-Cys(Trt)-OH, HATU, DIPEA, CH₂Cl₂, rt, 22 h (49% over two steps); (e) Et₂HN, CH₂Cl₂, rt, 2 h; (f) 20, HATU, DIPEA, CH₂Cl₂, rt, 18 h (57% over two steps); (g) TFA/thianisole/H₂O, rt, 2.5 h (13% after HPLC purification).

The configuration of the newly formed stereocenters at the bridge-head carbon atoms (C9a) in 11 and 12 (see Figure 5) was determined using the 2D ¹H ROESY experiment (see supplementary data for detailed ROESY spectra for 11 and 12). The known configurations of C6 (S for both 11 and 12) and C3 (R for 11 and S for 12) were used as prerequisites for determination of the configuration of C9a. The strong cross-peaks observed at δ 5.09/4.71 (H9a/H3), δ 5.09/1.73 (H9a/Hß arginine R²) and the medium strong cross-peak at δ 5.09/1.44 (H9a/Hγ arginine R²) observed in the 2D ROESY spectrum of 11 confirmed the (S)
configuration of C9a in 11. Moreover, the strong cross-peak observed at δ 5.00/4.72 (H9a/H3) in the 2D ROESY spectrum of 12 and the presence of only very weak cross-peaks at δ 5.00/1.64 (H9a/Hβa arginine R²) and δ 5.00/1.52 (H9a/Hβb arginine R²) confirmed the (R) configuration of C9a in 12.

The F building block required for 13 and 14 required a multi-step synthesis (Scheme 7). 2-Oxopiperidine derivative 28 was selectively reduced using freshly prepared Ca(BH₄)₂, and after an acidic work-up, alcohol 29 was isolated in good yield.²⁸ The alcohol was next taken through a carbodiimide-mediated dehydration to give α,β-unsaturated lactam 30.²⁸ Use of N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC) in refluxing toluene gave up to 73% yield, while DCC gave the product in quantitative yield. In both cases, Cul was added to activate the carbodiimide. Next, the lactam was Boc-protected to give 31 followed by Michael addition of triphenylmethane thiol to give racemic 32, with excellent yield in both steps. Finally, the Boc-protected lactam was hydrolyzed using LiOH to give racemic carboxylic acid 33 in high yield.

Scheme 7. Synthesis of building block 33. Reagents and conditions. (a) CaCl₂, NaBH₄, MeOH, 0 °C, 2 h, then rt, 16 h (76%); (b) DCC, CuI, toluene, 110 °C, 1 h (quant); (c) Boc₂O, Et₃N, DMAP, CH₂Cl₂/DMF, rt, 14 h (94%); (d) Ph₃CSH, Et₃N, CH₂Cl₂, rt, 14 h (95%); (e) 1M aq. LiOH, THF, rt, 1 h (94%).

Assembly of the linear precursors for 13 and 14 (Scheme 8) was carried out by Fmoc-deprotecting 23 and subsequent coupling of the resulting material with carboxylic acid 33, to give 34 as an inseparable mixture of diastereoisomers. Linear precursor 34 was globally deprotected and cyclized, and the amino group was guanidinylated to give 13 and 14.
Scheme 8. Synthesis of 13 and 14. Reagents and conditions. (a) Et₂HN, CH₂Cl₂, rt, 3 h; (b) 33, HBTU, DIPEA, CH₂Cl₂, rt, 17 h (85% over two steps); (c) TFA/thioanisole/H₂O, rt, 1.5 h; (d) H₃-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, rt, 72 h (7% for 13, 11% for 14, over two steps and after HPLC purification).

RP-HPLC analysis after guanidinylation showed two distinct peaks, which were separable by semi-prep RP-HPLC. NMR analysis of the two isolated products clearly showed that they were single diastereoisomers. For the two isolated products, only the configuration at C6 (S) was known, and the configuration of C3 and C9a was determined using the 2D ¹H ROESY experiment (see supplementary data). For both 13 and 14, strong cross-peaks between H9α and H3 (at δ 5.14/2.74 and at δ 4.66/2.38 for 13 and 14, respectively) were observed, indicating similar overall geometry to that of 11 and 12. The presence of a cross-peak between H9α and Hγ arginine R² was observed only in the ROESY spectrum of 13 suggesting that the configuration of C9α for this compound is S, whereas the configuration of C9α for the stereoisomer 14 is R. Since H9α and H3 are cis in both 13 and 14, the configuration at C3, which stems from the racemic carboxylic acid 33, is R in 13 and S in 14. It is interesting to note that S configuration of C9α in 11 and 13 leads to a downfield shift of H6 when compared with the analogous R isomer. Grimes et al. speculated that a solvent mediated hydrogen bond between the R¹-side chain amide NH and the ring carbonyl group could contribute to stabilizing a conformation in which the R¹ substituent is in an equatorial position, which favors the observed stereoselectivity of the cyclization. However, for the formation of 13 and 14, the absence of a R¹ amide NH did not influence on the stereoselectivity in the cyclization step.

3. CONCLUSIONS
In this work we have demonstrated that new scaffold-based tripeptidomimetic CXCR4 antagonists can be rationally designed from cyclopentapeptide CXCR4 antagonists. The bicyclic compounds reported herein represent an interesting class of new tripeptidomimetic CXCR4 antagonists, and although the prototype compounds showed moderate activity, they
serve as useful leads for further optimization. The peptidomimetic scaffold we have employed is constructed from three building blocks, each containing one of the pharmacophoric groups, and therefore allows for synthetic access to a range of target molecules. We envision that further SAR studies involving the three different binding groups will afford new and optimized CXCR4 antagonists.

4. EXPERIMENTAL SECTION

4.1. Chemistry.
All reagents and starting materials were purchased from Sigma-Aldrich and used as delivered unless otherwise stated. Cyclic peptide 2 was prepared as previously described.15 The preparation of 4–10 is described in the supplementary data. Anhydrous toluene, CH₂Cl₂ and THF were obtained from an anhydrous solvent delivery system (SDS-800 from mBraun) at the Department of Chemistry, University of Bergen. Analyses using thin layer chromatography were performed on Alugram® SIL G/UV₂₅₄ 0.20 mm layer plates from Machery-Nagel or on aluminum sheets with Merck silica gel (60 F₂₅₄). TLC plates were visualized using either ultraviolet light or by immersing the plate in 2% solution of ninhydrin in ethanol containing 10 drops of concentrated sulphuric acid pr 100 mL followed by heating. Purification by flash column chromatography was performed using J.T Baker Silica Gel or Merck 60 Kieselgel (230-400 mesh). All final compounds were purified using semi-preparative RP-HPLC eluting with mixtures of acetonitrile and H₂O (both containing 0.1% TFA). Fractions of equal purity were pooled and lyophilized. All tested compounds were analyzed by RP-HPLC and found to be of >95% purity (UV 220 nm).

4.1.1. (9H-Fluoren-9-yl)methyl (S)-(1-((2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-((2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (16)
HBTU (945 mg, 2.4 mmol) and DIPEA (0.66 mL, 3.7 mmol) were added to a stirred solution of Fmoc-Arg(Pbf)-OH (809 mg, 1.2 mmol) in dry DMF (3 mL) under an argon atmosphere. The mixture was stirred at room temperature for 30 min before a solution of 2-(naphthalen-2-yl)ethan-1-amine hydrochloride (518 mg, 2.5 mmol) in DMF (2 mL) was added drop-wise to the reaction mixture, and stirring continued for 20 h. The solvent was evaporated and the residue was partitioned between EtOAc (20 mL) and H₂O (10 mL). The organic phase was washed with H₂O (2 x 10 mL), 5% KHCO₃ (20 mL) and brine (20 mL), dried over MgSO₄,
filtered and evaporated. The crude product was purified by flash chromatography on silica gel (EtOAc/hexane; gradient 1:1 to 9:1) to give the title compound as a white solid (706 mg, 67%). \(R_f (\text{EtOAc/hexane 9:1}) = 0.23\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.75 – 7.67\) (m, 5H), 7.60 – 7.49 (m, 3H), 7.41 – 7.32 (m, 4H), 7.31 – 7.26 (m, 2H), 7.26 – 7.23 (m, 1H), 4.34 – 4.20 (m, 2H), 4.17 – 4.06 (m, 2H), 3.66 – 3.45 (m, 2H), 3.24 – 3.08 (m, 2H), 2.97 – 2.92 (m, 2H), 2.90 – 2.87 (m, 2H), 2.81 – 2.79 (m, 2H), 2.53 (s, 3H), 2.46 (s, 3H), 2.05 (s, 3H), 1.48 – 1.40 (m, 8H); \(^{13}\)C NMR (101 MHz, CD\(_3\)OD) \(\delta = 174.9, 173.4, 165.3, 160.3, 158.67, 158.66, 158.4, 145.7, 145.5, 143.0, 143.0, 139.9, 138.3, 135.4, 134.1, 134.0, 129.5, 129.20, 129.18, 129.0, 128.78, 128.74, 128.6, 127.4, 126.8, 126.6, 126.5, 121.3, 118.9, 88.1, 68.2, 62.0, 56.6, 44.3, 42.0, 39.3, 37.4, 36.9, 32.1, 30.9, 29.0, 21.3, 20.0, 18.8, 14.9, 13.0; HRMS (ESI): \(m/z [M + H]^+\) calcd for C\(_{46}\)H\(_{52}\)N\(_5\)O\(_6\)S: 802.3633; found: 802.3639.

4.1.2. (S)-5-Guanidino-2-(5-guanidinopentanamido)-N-(2-(naphthalen-2-yl)ethyl)pentanamide (3)

To a solution of the Fmoc-protected amine 16 (610 mg, 0.77 mmol) in DMF (6 mL) was added 2-ethanolamine (6 mL) and the mixture was stirred for 3 h at room temperature under HPLC monitoring. The solvent was removed in vacuo, the residue was dissolved in EtOAc, washed with H\(_2\)O (2 x 10 mL), saturated NaHCO\(_3\) (2 x 10 mL) and brine (15 mL). The solvent was evaporated and the crude product (500 mg) was used in the next step without further purification. HRMS (ESI): \(m/z [M + H]^+\) calcd for C\(_{31}\)H\(_{41}\)N\(_5\)O\(_4\)S: 580.2952; found: 580.2958.

HATU (590 mg, 1.5 mmol) and DIPEA (0.41 mL, 2.3 mmol) were added to a stirring solution of 5-((tert-butoxycarbonyl)amino)pentanoic acid (338 mg, 1.5 mmol) in dry DMF (3 mL) under an argon atmosphere. The mixture was stirred at room temperature for 30 min before a solution of the crude product from the previous step (451 mg, 0.78 mmol) in DMF (1.5 mL) was added drop wise to the reaction mixture, and stirring continued for 24 h. The reaction mixture was partitioned between EtOAc (30 mL) and H\(_2\)O (20 mL). The aqueous layer was extracted with EtOAc (2 x 15 mL) and the combined organic layer washed with 10% citric acid (15 mL), 5% KHCO\(_3\), (15 mL) and brine (15 mL), and dried over MgSO\(_4\). Filtration and removal of the solvent under reduced pressure gave the crude product (520 mg). Purification by flash chromatography (EtOAc/hexane; gradient 1:1 to pure EtOAc, followed by EtOAc/MeOH; gradient 9:1 to 8:2) afforded 17 as colorless foam (358 mg) which was judged to be of sufficient purity for the next step. HRMS (ESI): \(m/z [M + H]^+\) calcd for C\(_{41}\)H\(_{59}\)N\(_6\)O\(_7\)S: 779.4160; found: 779.4168.
The Boc-protected amine 17 (358 mg, 0.46 mmol) was dissolved in a mixture of TFA, TIS and H2O (95:2.5:2.5, 15 mL) and the resulting solution was stirred at room temperature. The reaction was monitored using analytical RP-HPLC and all starting material was found to be consumed after 2 h. The TFA solution was evaporated and the residue was precipitated by addition of cold diethyl ether (10 mL) and cooled overnight. The ether was decanted and the residue was dried in vacuo to give the crude product (403 mg), which was used in the next step without further purification. HRMS (ESI): \textit{m/z} [M + H]+ calcd for C23H34N6O2: 427.2816; found: 427.2816.

To a stirring solution of the crude primary amine (403 mg, 0.61 mmol) in DMF (3 mL) was added 1H-pyrazole-1-carboxamidine hydrochloride (453 mg, 3.0 mmol) and DIPEA (0.53 mL, 3.1 mmol) and the resulting mixture was stirred under nitrogen atmosphere for 48 h. The reaction was monitored using analytical RP-HPLC. After the solvent had been evaporated, the residue was precipitated by addition of cold diethyl ether, washed with ether, purified by preparative RP-HPLC, and lyophilized (129 mg, 21% over four steps). $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ = 7.59 – 7.51 (m, 3H), 7.42 (s, 1H), 7.24 – 7.10 (m, 3H), 4.01 (dd, $J$ = 8.3, 5.7, 1H), 3.39 – 3.23 (m, 2H), 2.90 (t, $J$ = 6.7, 2H), 2.80 (m, 2H), 2.73 (t, $J$ = 7.0, 2H), 2.00 (t, $J$ = 7.0, 2H), 1.51 – 1.09 (m, 8H); $^{13}$C NMR (101 MHz, CD$_3$OD): $\delta$ = 175.9, 174.3, 158.84, 158.81, 138.0, 135.2, 133.9, 129.3, 128.8, 128.54, 128.48, 127.2, 126.6, 54.7, 42.2, 42.0, 41.8, 36.7, 36.1, 30.4, 29.5, 26.4, 23.8; HRMS (ESI): \textit{m/z} [M + H]+ calcd for C24H36N8O2: 469.3034; found: 469.3033.

4.1.3. Methyl (N,N’-bis(tert-butoxycarbonyl)carbamimidoyl)glycinate (19)$^{26}$ To a slurry of glycine methyl ester hydrochloride (1.11 g, 8.8 mmol) in DMF (30.5 mL) was added N,N-di-Boc-1H-pyrazole-1-carboxamidine (1.02 g, 3.3 mmol) and DIPEA (1.1 mL, 6.3 mmol). The mixture was stirred overnight (14 h) at room temperature and turned into a yellow slurry. The reaction mixture was partitioned between EtOAc (25 mL) and H$_2$O (20 mL) and the aqueous layer was further extracted three times with EtOAc (25 mL), before the combined organic layers were dried over anhydrous MgSO$_4$. The solvent was evaporated under reduced pressure to give the crude product as a light yellow solid (1.4 g). Further purification using flash column chromatography (Hexanes/EtOAc, 3:1) gave the title compound as a colourless solid (932 mg, 85 %). R$_f$ (Hexanes/EtOAc, 3:1) = 0.27; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 11.44 (s, 1H), 8.87 (s, 1H), 4.25 (d, $J$ = 4.9, 2H), 3.78 (s, 3H), 1.51 (s, 9H), 1.50 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 170.2, 163.6, 156.3, 153.3, 83.7, 79.9,
52.8, 43.0, 28.6, 28.4; HRMS (ESI): m/z [M + H]^+ calcd for C_{14}H_{26}N_{3}O_{6}: 332.1822; found: 332.1821.

4.1.4. (N,N'-bis(tert-Butoxycarbonyl)carbamimidoyl)glycine (20)\textsuperscript{27}

LiOH·H₂O (166 mg, 4.0 mmol) was added to a solution of methyl ester 19 (330 mg, 1 mmol) in acetone/H₂O (6:1, 4.5 mL) at room temperature. TLC monitoring showed full consumption of the starting material after 1.5 h, and the reaction mixture was cooled to 0 °C before a 0.5 M aqueous HCl solution was added drop-wise until pH 3. The mixture was allowed to warm to room temperature and extracted with EtOAc (3 x 20 mL). Drying over anhydrous Na₂SO₄ and evaporation of the solvent gave the title compound as a colourless solid (312 mg, 99 %). R\textsubscript{f} (Hexanes/EtOAc/AcOH, 60:40:1) = 0.20; \textsuperscript{1}H NMR (400 MHz, CDCl₃) δ = 11.37 (s, 1H), 8.87 (s, 1H), 4.18 (s, 2H), 1.51 (s, 9H), 1.49 (s, 9H); \textsuperscript{13}C NMR (100 MHz, CDCl₃) δ = 171.7, 162.2, 157.0, 153.1, 84.4, 80.9, 44.3, 28.5, 28.3; HRMS (ESI): m/z [M + H]^+ calcd for C\textsubscript{13}H\textsubscript{23}N\textsubscript{3}O\textsubscript{6}: 318.1665; found: 318.1657.

4.1.5. 2,2-Dimethoxy-N-(2-(naphthalen-2-yl)ethyl)ethan-1-amine (22)

2-(Naphthalene-2-yl)ethyl-1-amine (21) (322 mg, 1.9 mmol) and bromoacetataldehyde dimethyl acetal (0.2 mL, 1.7 mmol) were dissolved in dry THF (4 mL), and the mixture was heated at a gentle reflux for 24 h. After the solvent was evaporated, the residue was dissolved in CH₂Cl₂ (20 mL) and the organic phase was washed with saturated NaHCO₃ (2 x 10 mL) and brine. The mixture was concentrated and purified by flash chromatography on silica gel (EtOAc) to give the title compound (130 mg, 30%) as yellow oil. R\textsubscript{f} (EtOAc) = 0.14; \textsuperscript{1}H NMR (400 MHz, CDCl₃): δ = 7.78 (t, J = 8.6, 3H), 7.64 (s, 1H), 7.49 – 7.37 (m, 2H), 7.33 (d, J = 8.4, 1H), 4.45 (t, J = 5.4, 1H), 3.34 (s, 6H), 2.96 (s, 4H), 2.78 (d, J = 5.4, 2H); \textsuperscript{13}C NMR (100 MHz, CDCl₃): δ = 137.7, 133.8, 132.4, 128.3, 127.9, 127.7, 127.5, 127.2, 126.2, 125.5, 104.2, 54.2, 51.39, 51.36, 36.8; HRMS (ESI): m/z [M + H]^+ calcd for C\textsubscript{16}H\textsubscript{22}NO\textsubscript{2}: 260.1645; found: 260.1646.

4.1.6. (9H-Fluoren-9-yl)methyl (S)-(1-((2,2-dimethoxyethyl)(2-(naphthalen-2-yl)ethyl)amino)-1-oxo-5-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (23)

HATU (183 mg, 0.48 mmol) and DIPEA (84 µL, 0.48 mmol) were added to a stirred solution of Fmoc-Arg(Pbf)-OH (292 mg, 0.45 mmol) in dry DMF (1.2 mL) under an argon atmosphere. The mixture was stirred at room temperature for 30 min before amine 22 (125
mg, 0.48 mmol) in DMF (0.6 mL) was added drop-wise to the reaction mixture, and stirring continued for 16 h. The reaction mixture was partitioned between H₂O (10 mL) and EtOAc (20 mL). The organic phase was further washed with H₂O (2 x 10 mL) and brine (2 x 10 mL), dried over MgSO₄, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (EtOAc/hexane 8.5:1.5) to give the title compound as white foam (334 mg, 83 %, retains EtOAc). Rf (EtOAc/hexane 8.5:1.5) = 0.28; ¹H NMR and ¹³C NMR gave no useful information due to rotamers; HRMS (ESI): m/z [M + H]⁺ calcd for C₅₀H₆₀N₅O₈S: 890.4157; found: 890.4166; m/z [M + Na]⁺ calcd for C₅₀H₅₉N₅O₈SNa: 912.3977; found: 912.3976.

4.1.7. (9H-Fluoren-9-yl)methyl ((7S,10R)-3-methoxy-5-(2-(naphthalen-2-yl)ethyl)-6,9-dioxo-7-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-13,13,13-triphenyl-2-oxa-12-thia-5,8-diazatridecan-10-yl)carbamate (24)

Et₂NH (1.5 mL) was added to a solution of the Fmoc-protected amine 23 (261 mg, 0.29 mmol) in CH₂Cl₂ (3 mL) and the mixture was stirred at room temperature for 2 h with TLC monitoring. The solvent was removed under reduced pressure, to give a light yellow foam. The crude product (230 mg) was used directly in the next step.
To a solution of crude Fmoc-deprotected 23 (0.29 mmol) in CH₂Cl₂ (2 mL), were added Fmoc-Cys(Trt)-OH (183 mg, 0.31 mmol), HATU (112 mg, 0.30 mmol) and DIPEA (0.14 mL, 0.8 mmol), and the mixture was stirred for 22 h at room temperature. After evaporation of the solvent, the residue was partitioned between EtOAc (15 mL) and H₂O (50 mL). The aqueous layer was extracted with EtOAc (2 x 15 mL), and the combined organic layer was washed with 1M KHSO₄ (30 mL), H₂O (30 mL), saturated NaHCO₃ (30 mL), brine (30 mL) and was dried over anhydrous MgSO₄. Removal of the solvent under reduced pressure gave the crude product as an orange colored foam (419 mg). Purification by flash column chromatography (EtOAc/hexanes, 8:2) gave 24 as a colorless foam (194 mg, 49 % over 2 steps). ¹H NMR showed that the product retained EtOAc, and the yield is adjusted accordingly. Rf (EtOAc/hexane, 8:2) = 0.16; ¹H NMR and ¹³C NMR gave no useful information due to rotamers; HRMS (ESI): m/z [M + Na]⁺ calcd for C₇₂H₇₈N₆O₉S₂Na: 1257.5169; found: 1257.5172.

4.1.8. tert-Butyl ((7S,10R)-3-methoxy-19,19-dimethyl-5-(2-(naphthalen-2-yl)ethyl)-6,9,12,17-tetraoxo-7-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-
**ylsulfonyl)guanidino)-propyl)-10-(tritylthiomethyl)-2,18-dioxa-5,8,11,14,16-pentaazaicosan-15-ylidene)carbamate (25)**

Et$_2$NH (0.73 mL) was added to a solution of the Fmoc-protected amine 24 (181 mg, 0.15 mmol) in CH$_2$Cl$_2$ (1.5 mL) at room temperature. TLC monitoring showed no sign of the starting material after 2 h and the solvent was removed under reduced pressure to give a light yellow foam. The crude product (183 mg) was used directly in the next step. HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{57}$H$_{69}$N$_6$O$_7$S$_2$: 1013.4669; found: 1013.4672.

To a solution of carboxylic acid 20 (183 g, 0.31 mmol) in CH$_2$Cl$_2$ (1 mL), were added HATU (60 mg, 0.16 mmol), DIPEA (0.07 mL, 0.40 mmol) and Fmoc-deprotected 24 (65 mg, 0.2 mmol) and the resulting yellow reaction mixture was allowed to stir for 18 h at room temperature. The solvent was evaporated and the residue was partitioned between EtOAc (10 mL) and H$_2$O (10 mL). The aqueous layer was extracted with EtOAc (2 x 10 mL), and the combined organic layer was washed with 1M KHSO$_4$ (20 mL), H$_2$O (20 mL), saturated NaHCO$_3$ (20 mL), brine (20 mL) and was dried over anhydrous MgSO$_4$. Removal of the solvent under reduced pressure gave the crude product as a yellow foam (203 mg). Purification by flash column chromatography (EtOAc/hexanes, 9:1) gave the title compound as a light yellow foam (101 mg, 57 % over 2 steps). $^1$H NMR showed that the product retained EtOAc, and the yield is adjusted accordingly. R$_f$ (EtOAc/hexanes, 9:1) = 0.21; $^1$H NMR and $^{13}$C NMR gave no useful information due to rotamers; HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{70}$H$_{90}$N$_9$O$_{12}$S$_2$: 1312.6145; found: 1312.6146.

**4.1.9. 2-Guanidino-N-((3R,6S,9aS)-6-(3-guanidinopropyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2H,6H-pyrazino[2,1-b][1,3]thiazin-3-yl)acetamide (11)**

Linear precursor 25 (92 mg, 0.07 mmol) was dissolved in a mixture of TFA, thioanisole and distilled H$_2$O (90:5:5, 8 mL) and stirred for 2.5 h at room temperature. After evaporation of the solvent under reduced pressure the brown residue was cooled to 0 °C. Addition of cold ether resulted in precipitation and the crude solid was purified by preparative RP-HPLC, and lyophilized to give 11 as a fluffy white solid (7.2 mg, 13%). $^1$H NMR (600 MHz, CD$_3$OD): $\delta$ = 7.82 – 7.79 (m, 3H), 7.70 (s, 1H), 7.47 – 7.41 (m, 3H), 5.09 (dd, $J =$ 7.4, 4.0, 1H), 4.95 (dd, $J =$ 9.3, 6.1, 1H), 4.71 (t, $J =$ 8.8, 1H), 4.03 – 3.97 (m, 3H), 3.81 (dd, $J =$ 13.3, 7.5, 1H), 3.64 (dd, $J =$ 13.4, 4.0, 1H), 3.53 (m, 1H), 3.11 – 3.01 (m, 6H), 1.79 – 1.66 (m, 2H), 1.48 – 1.40 (m, 2H); $^{13}$C NMR (150.9 MHz, CD$_3$OD) $\delta$ = 170.0, 169.6, 168.9, 159.5, 158.5, 137.3, 135.1, 133.9, 129.3, 128.7, 128.5, 128.44, 128.40, 127.2, 126.7, 57.3, 52.27, 52.26, 51.4, 49.5, 44.7, 41.6, 34.4, 29.8, 27.1, 26.3; HRMS (ESI): m/z [M + 2H]$^{2+}$ (z = 2) calcd for C$_{26}$H$_{37}$N$_9$O$_3$S:
227.6365; found: 227.6359; HRMS (ESI): m/z [M + H]⁺ calcd for C_{26}H_{36}N_{9}O_{3}: 554.2656; found: 554.2659.

4.1.10. (9H-fluoren-9-yl)methyl ((7S,10S)-3-methoxy-5-(2-(naphthalen-2-yl)ethyl)-6,9-dioxo-7-(3-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-13,13,13-triphenyl-2-oxa-12-thia-5,8-diazatridecan-10-yl)carbamate (26)

Et₂NH (7 mL) was added to a solution of the Fmoc-protected amine 23 (308 mg, 0.33 mmol) in CH₂Cl₂ (7 mL) and the reaction was stirred for 3 h at room temperature with HPLC monitoring. The solvent was removed under reduced pressure and the crude product (320 mg) was used directly in the next step. HRMS (ESI): m/z [M + H]⁺ calcd for C_{35}H_{50}N_{5}O_{6}: 668.3476; found: 668.3488.

Fmoc-D-Cys(Trt)-OH (364 mg, 0.62 mmol), HATU (236 mg, 0.62 mmol) and DIPEA (0.25 mL, 1.4 mmol) were dissolved in CH₂Cl₂ (2 mL) and the resulting mixture was stirred for 30 min at room temperature, before a solution of crude Fmoc-deprotected 23 (320 mg, 0.48 mmol) in CH₂Cl₂ (3 mL) was added. The reaction mixture was stirred for 24 h at room temperature under anhydrous conditions (CaCl₂). After evaporation of the solvent the residue was partitioned between EtOAc (20 mL) and H₂O (40 mL). The aqueous layer was extracted with EtOAc (2 x 15 mL), and the combined organic layer was washed with 1M KHSO₄ (30 mL), H₂O (30 mL), saturated NaHCO₃ (30 mL), brine (30 mL) and was dried over anhydrous MgSO₄. Removal of the solvent under reduced pressure gave the crude product as a light red colored foam (710 mg). Purification by flash column chromatography (EtOAc/hexanes, 8:2) gave the title compound as a colorless foam (237 mg, 45% over two steps). Rf (EtOAc/hexane, 8:2) = 0.19; HRMS (ESI): m/z [M + H]⁺ calcd for C_{72}H_{78}N_{6}O_{9}S₂: 1235.5344; found: 1235.5334.

4.1.11. tert-Butyl ((7S,10S)-3-methoxy-19,19-dimethyl-5-(2-(naphthalen-2-yl)ethyl)-6,9,12,17-tetraoxo-7-(3-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)-propyl)-10-(tritylthiomethyl)-2,18-dioxa-5,8,11,14,16-pentaazaicosan-15-ylidene)carbamate (27)

Et₂NH (6 mL) was added to a solution of the Fmoc-protected amine 26 (237 mg, 0.19 mmol) in CH₂Cl₂ (6 mL) and the reaction was stirred for 3 h at room temperature with HPLC monitoring. The solvent was removed under reduced pressure and the crude product (277...
mg), was used directly in the next step. HRMS (ESI): m/z [M + H]^+ calcd for C_{57}H_{69}N_{6}O_{7}S_{2}: 1013.4664; found: 1013.4663

To a solution of carboxylic acid 20 (150 mg, 0.47 mmol) in CH_{2}Cl_{2} (2 mL), were added HATU (156 mg, 0.41 mmol) and DIPEA (0.15 mL, 0.82 mmol). The mixture was stirred at room temperature under anhydrous environment (CaCl_{2}) for 15 min before a solution of the Fmoc-deprotected amine 26 (277 mg, 0.27 mmol) in CH_{2}Cl_{2} (2 mL) was added drop-wise to the reaction mixture, and stirring continued for 24 h. After evaporation of the solvent the residue was partitioned between EtOAc (20 mL) and H_{2}O (30 mL). The aqueous layer was extracted with EtOAc (2 x 15 mL), and the combined organic layer was washed with 1M KHSO_{4} (30 mL), H_{2}O (30 mL), saturated NaHCO_{3} (30 mL), brine (30 mL), and was dried over anhydrous MgSO_{4}. Removal of the solvent under reduced pressure gave the crude product (352 mg). Purification by flash column chromatography (EtOAc/hexanes, 9:1) gave the title compound (93 mg, 37% over 2 steps). R_{f} (EtOAc/hexane, 9:1) = 0.45; NMR analyses did not give useful information due to formation of rotamers; HRMS (ESI): m/z [M + H]^+ calcd for C_{70}H_{89}N_{9}O_{12}S_{2}: 1312.6145; found: 1312.6141.

4.1.12. 2-guanidino-N-((3S,6S,9aS)-6-(3-guanidinopropyl)-8-(2-(naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2H,6H-pyrazino[2,1-b][1,3]thiazin-3-yl)acetamide (12)

Linear precursor 27 (76 mg, 0.06 mmol) was dissolved in a mixture of TFA, thioanisole and distilled H_{2}O (90:5:5, 7 mL) and stirred for 2 h. After evaporation of the solvent under reduced pressure the brown residue was cooled to 0 °C. Addition of cold ether resulted in precipitation and the crude product was purified by preparative RP-HPLC, and lyophilized to give the title compound as a fluffy white solid (1.5 mg, 3%). ^1H NMR (600 MHz, CD_{3}OD): δ = 7.84 – 7.78 (m, 3H), 7.70 (s, 1H), 7.46 – 7.39 (m, 3H), 5.00 (dd, J = 11.0, 5.4, 1H), 4.72 (dd, J = 11.9, 6.3, 1H), 4.64 (t, J = 8.0, 1H), 4.15 – 4.08 (m, 1H), 3.99 (q, J = 29.4, 17.3, 2H), 3.70 – 3.55 (m, 3H), 3.36 (dd, J = 10.6, 6.3, 1H), 3.12 – 3.04 (m, 2H), 3.01 – 2.96 (m, 1H), 2.90 – 2.85 (m, 1H), 2.73 (t, J = 11.3, 1H), 1.67 – 1.58 (m, 1H), 1.56 – 1.49 (m, 1H), 1.31 – 1.24 (m, 2H); ^13C NMR (150.9 MHz, CD_{3}OD) δ = 170.5, 169.9, 169.3, 159.4, 158.4, 137.2, 135.0, 133.9, 129.3, 128.7, 128.6, 128.5, 128.4, 127.4, 126.8, 57.6, 52.8, 51.4, 48.9, 48.7, 44.6, 41.4, 34.7, 30.7, 28.5, 26.3; HRMS (ESI): m/z [M + H]^+ calcd for C_{26}H_{33}N_{9}O_{3}S; 554.2656; found: 554.2659.
4.1.13. 3-(Hydroxymethyl)piperidin-2-on (29)\textsuperscript{28}

To a stirred suspension of ethyl 2-oxopiperidine-3-carboxylate (28) (1.00 g, 5.8 mmol) and anhydrous CaCl\textsubscript{2} (702 mg, 6.3 mmol) in dry MeOH (12 mL) in an ice/H\textsubscript{2}O bath, NaBH\textsubscript{4} (501 mg, 13 mmol) was added in one portion, and stirring was continued for 2 h at 0 °C. After 16 h of additional stirring at room temperature, the solvent was evaporated, and a 3N aqueous citric acid solution was added in small portions until all solid material was dissolved (pH = 2-3). The solution was extracted with CH\textsubscript{2}Cl\textsubscript{2} (6 x 50 mL), dried over Na\textsubscript{2}SO\textsubscript{4} and filtered. Evaporation of the solvent gave the crude product (643 mg), which was purified by flash column chromatography on silica gel (5 % MeOH in EtOAc) to give the title compound as white solid (573 mg, 76 %). \( R_f (\text{EtOAc}) = 0.11; \) \(^1\text{H} \) NMR (400 MHz, CDCl\textsubscript{3}): \( \delta = 5.99 \) (bs, 1H), 4.07 (d, \( J = 8.5, 1\text{H} \)), 3.81 –3.60 (m, 2H), 3.39 –3.21 (m, 2H), 2.56 –2.42 (m, 1H), 1.97 –1.71 (m, 3H), 1.56 –1.39 (m, 1H); \(^{13}\text{C} \) NMR (100 MHz, CDCl\textsubscript{3}, 25°C): \( \delta = 176.2, 64.9, 43.2, 42.0, 24.1, 22.3 \); HRMS (ESI): \( m/z [M + H]^+ \) calcd for C\textsubscript{6}H\textsubscript{12}NO: 130.0868; found: 130.0850.

4.1.14. 3-Methylene-piperidin-2-one (30)\textsuperscript{28}

\( N,N' \)-Dicyclohexylcarbodiimide (DCC) (346 mg, 1.7 mmol) was added to a stirring solution of alcohol 29 (167 mg, 1.3 mmol) in dry toluene (2 mL). The mixture was heated at 110 °C (oil bath) and CuI (23 mg, 0.12 mmol) was added and the mixture was stirred for 70 min before it was cooled to room temperature, after which H\textsubscript{2}O (1.6 mL) was added and stirring continued for 1 h. Et\textsubscript{2}O (3.4 mL) was added, and the mixture was filtered. The aqueous phase was separated and extracted four times with 30 mL portions of CH\textsubscript{2}Cl\textsubscript{2}. The combined extracts were dried over K\textsubscript{2}CO\textsubscript{3}, filtered and evaporated to give the title compound as white solid (144 mg, 100 %). \( R_f (\text{EtOAc}) = 0.17; \) \(^1\text{H} \) NMR (400 MHz, CDCl\textsubscript{3}): \( \delta = 6.91 \) (bs, 1H), 6.21 (bs, 1H), 5.37 – 5.26 (m, 1H), 3.40 (td, \( J = 6.2, 2.6, 2\text{H} \)), 2.67 – 2.51 (m, 2H), 1.95 – 1.79 (m, 2H); \(^{13}\text{C} \) NMR (100 MHz, CDCl\textsubscript{3}, 25°C): \( \delta = 166.6, 138.1, 122.5, 43.2, 30.4, 23.7 \); HRMS (ESI): \( m/z [M + H]^+ \) calcd for C\textsubscript{6}H\textsubscript{10}NO: 112.0762; found: 112.0779.

4.1.15. tert-Butyl 3-methylene-2-oxopiperidine-1-carboxylate (31)

Et\textsubscript{3}N (1.2 mL, 8.8 mmol) and DMAP (1.07 g, 8.8 mmol) were added to a stirring solution of lactam 30 (971 mg, 8.7 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2}/DMF (10:1, 27 mL). Boc\textsubscript{2}O (3.83 g, 17 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (12 mL) was added drop-wise, and the reaction mixture was stirred at room temperature for 14 h. The mixture was concentrated and purified by flash column chromatography on silica gel (EtOAc/hexane 1:4) to give the title compound (1.72 g, 94 %)
as transparent thick oil. \( R_f \) (EtOAc/hexane 1:4) = 0.34; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 6.33 \) (d, \( J = 1.4 \), 1H), 5.41 (d, \( J = 0.7 \), 1H), 3.78 – 3.67 (m, 2H), 2.58 (t, \( J = 6.1 \), 2H), 1.95 – 1.84 (m, 2H), 1.55 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 164.8, 153.3, 138.8, 125.1, 83.1, 46.9, 29.5, 28.2, 22.8 \); HRMS (ESI): \( m/z \) [M + Na]\(^+\) calcd for C\(_{11}\)H\(_{17}\)NO\(_3\)Na: 234.1102; found: 234.1101.

4.1.16. tert-Butyl 2-oxo-3-(tritylthiomethyl)piperidine-1-carboxylate (32)
To a stirred solution of lactam 31 (1.65 g, 7.8 mmol) in dry CH\(_2\)Cl\(_2\) (33 mL) was added Ph\(_3\)CSH (2.16 g, 7.8 mmol) and Et\(_3\)N (1.1 mL, 7.8 mmol) and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was washed with H\(_2\)O, concentrated and purified by flash column chromatography on silica gel (hexane/EtOAc 8:2) to give the title compound as white foam (3.60 g, 95 %). \( R_f \) (hexane/EtOAc 4:1) = 0.18; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 7.45 – 7.20 \) (m, 15H), 3.76 – 3.66 (m, 1H), 3.34 (ddd, \( J = 12.9, 7.6, 5.1 \), 1H), 2.88 (ddd, \( J = 12.7, 4.6, 1 \)H), 2.28 (ddd, \( J = 12.7, 8.6, 1 \)H), 2.09 – 1.97 (m, 1H), 1.95 – 1.81 (m, 1H), 1.75 – 1.65 (m, 2H), 1.49 (s, 9H), 1.33 – 1.26 (m, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 172.8, 152.9, 145.0, 129.9, 128.1, 126.9, 83.2, 67.1, 45.1, 43.6, 33.0, 28.2, 25.5, 21.7 \); HRMS (ESI): \( m/z \) [M + Na]\(^+\) calcd for C\(_{30}\)H\(_{33}\)NO\(_4\)SNa: 510.2073; found: 510.2070.

4.1.17. 5-(tert-Butoxycarbonylamino)-2-((tritylthio)methyl)pentanoic acid (33)
To a stirred solution of lactam 32 (3.60 g, 7.4 mmol) in THF (37 mL), was added LiOH (1.0 M, 15 mL, 15 mmol). The reaction was monitored by TLC and all starting material was consumed after 1 h. The THF was evaporated and the aqueous residue was acidified to pH 4 with 10% citric acid. The mixture was extracted with CH\(_2\)Cl\(_2\) (4 x 50 mL), dried over MgSO\(_4\), filtered and evaporated to give the title compound as white foam (3.52 g, 94%). \( R_f \) (hexane/EtOAc 6:4) = 0.20; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 7.46 – 7.17 \) (m, 15H), 4.47 (bs, 1H), 2.97 (bs, 2H), 2.61 (ddd, \( J = 12.5, 8.1 \) Hz, 1H), 2.22 (ddd, \( J = 12.6, 6.1 \) Hz, 1H), 2.11 – 1.99 (m, 1H), 1.50 – 1.33 (s and m, 11H), 1.26 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 179.8, 156.2, 144.8, 129.9, 128.2, 127.0, 79.5, 67.2, 44.9, 40.3, 33.3, 29.0, 28.6, 27.5 \); HRMS (ESI): \( m/z \) [M + Na]\(^+\) calcd for C\(_{30}\)H\(_{33}\)NO\(_4\)SNa: 528.2184; found: 528.2174.
4.1.18. tert-Butyl ((SR)-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-4-((tritylthio)methyl)pentyl)carbamate (34)

Et₂HN (1.9 mL, 18 mmol) was added to a stirring solution of 23 (334 mg, 3.8 mmol) in CH₂Cl₂ (3.9 mL) at room temperature. The reaction was monitored by TLC and deemed completed after 3 h. The mixture was evaporated to dryness and the residue used in the following step.

To a stirred solution of carboxylic acid 33 (182 mg, 0.36 mmol) in dry CH₂Cl₂ (2.1 mL), were added HBTU (142 mg, 0.38 mmol) and DIPEA (66 µL, 0.38 mmol) and the mixture was stirred for 30 min. Fmoc-deprotected amine 23 (250 mg out of 344 mg crude product, 0.38 mmol) in dry CH₂Cl₂ (2.1 mL) was added and stirring continued for 17 h. The solvent was evaporated to give red foam, which was partitioned between EtOAc (20 mL) and H₂O (10 mL). The organic phase was separated and washed with 5% KHSO₄ (10 mL) and brine (10 mL). The organic phase was further concentrated and purified by flash chromatography on silica gel (EtOAc/hexane 3:1) to give an inseparable mixture of the two diastereoisomers of 34 as white foam (352 mg, 85 %). The product retained EtOAc and the yield is adjusted accordingly. Rf (EtOAc/hexane, 3:1) = 0.29; HRMS (ESI): m/z [M + H]+ calcd for C₆₅H₈₃N₆O₉S₂: 1155.5658; found: 1155.5667; HRMS (ESI): m/z [M + Na]+ calcd for C₆₅H₈₂N₆O₉S₂Na: 1177.5477; found: 1177.5474.

4.1.19. 1,1'-(((3R,6S,9aS)-8-(2-(Naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2H,6H-pyrazino[2,1-b][1,3]thiazine-3,6-diyl)bis(propane-3,1-diyl))diguanidine (13)

The diastereoisomeric mixture of linear precursor 34 (925 mg, 0.8 mmol) was dissolved in a mixture of TFA, thioanisole and H₂O (90:5:5, 28 mL) and the resulting mixture was stirred at room temperature for 90 min. The TFA mixture was evaporated and the crude product was precipitated by addition of cold diethyl ether. The ether was drained off and the residue was dried in vacuo to give the crude product as a reddish solid (422 mg). The crude product (210 mg, approx. 0.43 mmol) in dry DMF (3.5 mL) was added 1H-pyrazole-1-carboxamidine hydrochloride (92 mg, 0.63 mmol) and DIPEA (0.11 mL, 0.63 mmol), and the mixture was stirred under argon atmosphere for 72 h during which the reaction was monitored by RP-HPLC. Diethyl ether (30 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 (230 mg). The crude product was purified by preparative HPLC to give the title compound as a fluffy white solid.
(11 mg, 7%). $^1$H NMR (600 MHz, CD$_3$OD) $\delta$ = 7.82 – 7.77 (m, 3H), 7.69 (s, 1H), 7.47 – 7.40 (m, 3H), 5.14 (dd, $J$ = 5.4, 4.6, 1H), 4.00 (dt, $J$ = 13.6, 7.7, 1H), 3.70 (dd, $J$ = 13.9, 4.0, 1H), 3.58 – 3.51 (m, 2H), 3.17 (t, $J$ = 7.0, 2H), 3.10 – 3.98 (m, 5H), 2.78 – 2.73 (m, 1H), 2.58 (t, $J$ = 12.0, 1H), 1.91 – 1.85 (m, 1H), 1.68 – 1.56 (m, 4H), 1.46 – 1.41 (m, 1H), 1.40 – 1.35 (m, 3H); $^{13}$C NMR (150.9 MHz, CD$_3$OD) $\delta$ = 173.8, 169.4, 158.7, 158.5, 137.3, 135.0, 133.8, 129.3, 128.7, 128.51, 128.49, 128.45, 127.2, 126.7, 56.6, 52.9, 50.9 (HSQC), 49.3 (HSQC), 43.8, 42.4, 41.6, 34.4, 30.2, 28.9, 28.6, 27.5, 26.2; HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{27}$H$_{39}$N$_8$O$_2$S: 539.2911; found: 539.2906.

4.1.20. 1,1'–(((3$^S$,6$^S$,9a$^R$)-8-(2-(Naphthalen-2-yl)ethyl)-4,7-dioxohexahydro-2H,6H-pyrazino[2,1-b][1,3]thiazine-3,6-diyl)bis(propane-3,1-diyl))diguanidine (14)

Bicycle 14 was prepared as described for 13. The crude was purified by preparative HPLC to give the title compound as a fluffy white solid (16 mg, 11%). $^1$H NMR (600 MHz, CD$_3$OD) $\delta$ = 7.84 – 7.80 (m, 2H), 7.78 (d, $J$ = 7.7, 1H), 7.67 (d, 1H), 7.47 – 7.43 (m, 3H), 4.68 – 4.62 (m, 2H), 4.08 – 4.02 (m, 1H), 3.68 (dt, $J$ = 13.6, 5.9, 1H), 3.60 (d, $J$ = 9.1, 2H), 3.19 – 3.14 (m, 3H), 3.08 (t, $J$ = 6.7, 2H), 3.00 – 2.95 (m, 1H), 2.94 – 2.90 (m, 1H), 2.55 (t, $J$ = 11.4, 1H), 2.41 – 2.36 (m, 1H), 1.85 – 1.79 (m, 1H), 1.65 – 1.57 (m, 3H), 1.53 – 1.46 (m, 1H), 1.45 – 1.39 (m, 1H), 1.33 – 1.25 (m, 2H); $^{13}$C NMR (150.9 MHz, CD$_3$OD) $\delta$ = 174.1, 170.4, 158.7, 158.5, 137.3, 134.9, 133.8, 129.3, 128.73, 128.70, 128.42, 128.41, 127.4, 126.8, 57.1, 53.7, 49.0 (HSQC), 48.9 (HSQC), 42.8, 42.4, 41.5, 34.6, 30.7, 29.9, 28.8, 27.7, 26.3; HRMS (ESI): m/z [M + H]$^+$ calcd for C$_{27}$H$_{39}$N$_8$O$_2$S: 539.2911; found: 539.2905.

4.2. Molecular modeling.

The conformational search for scaffolds A and A' was performed using the MacroModel application as integrated in Schrödinger Suite 2012, running on a Dell Precision 390 workstation. The OPLS_2005 force field and the GB/SA solvent model for water were used for the energy calculations. The Truncated Newton Conjugate Gradient (TNCG) algorithm was used for the energy minimizations, with maximum 500 iterations and a convergence threshold of 0.012 kcal/mol×Å (0.05 kJ/mol×Å). The Monte Carlo Multiple Minimum (MCMM) search algorithm was used for torsional sampling (1000 steps), and the energy cutoff was set to 5 kcal/mol (21 kJ/mol) above the lowest energy conformation. Mirror image conformations were retained, and redundant conformations were eliminated by using an RMSD cutoff (all atoms) of 0.5 Å. For both scaffolds, this resulted in four low-energy conformations, which were superimposed on the backbone of our previously reported 3D
pharmacophore model for cyclopentapeptide CXCR4 antagonists using five atoms: the α-carbon atoms of Arg\(^1\), Arg\(^2\), and 2-Nal\(^3\) and the β-carbon atoms of Arg\(^1\) and Arg\(^2\).

4.3. Biological Studies.

4.3.1. Transfections and tissue culture.

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) with Glutamax (Invitrogen, UK) supplemented with 10% fetal bovine serum (FBS), 180 units/mL penicillin and 45 µg/mL streptomycin (PenStrep) at 37 °C in a 10% CO\(_2/90\%\) humidified atmosphere. Transfection of cells was carried out by the calcium phosphate precipitation method.\(^{30,31}\) Briefly, plasmid DNA (20 µg of receptor cDNA and 30 µg of the chimeric G\(\alpha_i\) to G\(\alpha_q\) signal-converting G protein G\(\alpha_{\Delta6qi4myr}\)) were mixed with TE-buffer (10 mM Tris-HCl, 2 mM Na\(_2\)EDTA, pH 7.5) and 30 µL calcium chloride (2 M) to a total volume of 480 µL, and then added to the same amount of HEPES buffered saline (280 mM NaCl, 50 mM HEPES, 1.5 mM Na\(_2\)HPO\(_4\), pH 7.2). Precipitation was allowed for 45 min at room temperature, after which the precipitate together with 300 µL chloroquine (2 mg/mL) in 10 mL culture media was added to the 6 \(\times\) 10\(^6\) COS-7 cells seeded the day before. Transfection was stopped after 5 h by replacing media, and cells were incubated overnight.

4.3.2. Functional assay.

The potency was measured using a scintillation proximity-based inositol-phosphate accumulation assay (SPA-IP).\(^{32}\) In brief, one day after transfection COS-7 cells (0.35 \(\times\) 10\(^5\) cells/well) were incubated for 24 h with \[^{3}\text{H}\]myo-inositol (5 µL/mL, 2 µCi/mL) in 0.1 mL of growth medium per well in a 96-well plate. The following day, cells were washed twice in PBS and were incubated in 0.1 mL of Hank's balanced salt solution (Invitrogen, UK) supplemented with 10 mM LiCl at 37 °C in the presence of various concentrations of ligands for 90 min. Cells were extracted by addition of 50 µL of 10 mM formic acid to each well, followed by incubation on ice for 30–60 min. The \[^{3}\text{H}\]inositol-phosphates in the formic acid cell lysates were quantified by Ysi-poly-D-Lys coated SPA beads. Briefly, 35 µL of cell extract was mixed with 80 µL of a SPA bead suspension (12.5 µg/µL in H\(_2\)O) in a PicoPlate-96 white plate. Plates were sealed, agitated for at least 30 min and centrifuged (5 min, 1500 rpm). SPA beads were allowed to settle and react with the extract for 8 h before radioactivity was determined using a Packard Top Count NXT™ scintillation counter (PerkinElmer, MA, USA). All determinations were made in duplicate. This readout has earlier been used effectively for CXCR4 and other chemokine receptors.\(^{13,33}\)
SUPPLEMENTARY DATA
Experimental procedures for 4–10 and relevant sections of ROESY spectra for compounds 11–14 can be found in the online version, at http://

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ABBREVIATIONS
2-Nal, L-3-(2-naphthyl)alanine; CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor 4; DIPEA, N,N-diisopropylethylamine; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate; HBTU, 1-[bis(dimethylamino)methylene]-1H-benzotriazolium-3-oxide hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoroisopropanol; HOBt, 1-hydroxybenzotriazole; Pbf, 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl; PyBOP, (benzotriazol-1-yl oxy)tri pyrrolidinophosphonium hexafluorophosphate; SPPS, solid phase peptide synthesis; TIS, triisopropylsilane; Trt, triphenylmethyl.

REFERENCES AND NOTES
20. In previous publications these structures have been denoted as being 3,8,10-trisubstituted.