Journal of Medicinal Chemistry

Brief Article

Rational Design of Conformationally Constrained Cyclopentapeptide Antagonists for C-X-C Chemokine Receptor 4 (CXCR4)

Jignesh Mungalpara, Stefanie Thiele, Øystein Eriksen, Johann Eksteen, Mette M. Rosenkilde, and Jon Våbenø

J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 09 Oct 2012

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Rational Design of Conformationally Constrained Cyclopentapeptide Antagonists for C-X-C Chemokine Receptor 4 (CXCR4)

Jignesh Mungalpara,[†] Stefanie Thiele,[‡] Øystein Eriksen,[†] Johann Eksteen,[§] Mette M. Rosenkilde,[‡] and Jon Våbenø*,[†]

[†]Department of Pharmacy, Faculty of Health Sciences, University of Tromsø, Breivika, NO-9037 Tromsø, Norway

[‡]Laboratory for Molecular Pharmacology, Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark

[§]Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Breivika, NO-9037 Tromsø, Norway; Lytix Biopharma, Tromsø Science Park AS, P.O. Box 6447, NO-9294 Tromsø, Norway

Supporting Information Placeholder

ABSTRACT: In the absence of an experimentally determined binding mode for the cyclopentapeptide CXCR4 antagonists, we have rationally designed conformationally constrained analogs to further probe the small peptide binding pocket of CXCR4. Two different rigidification strategies were employed, both resulting in highly potent ligands (compounds **9** and **13**). The information provided by this cyclopentapeptide ligand series will be very valuable in the development of novel peptidomimetic CXCR4 antagonists.

INTRODUCTION

The C-X-C chemokine receptor 4 (CXCR4), which is activated by chemokine (C-X-C motif) ligand 12 (CXCL12),[1] has attracted a lot of interest due to its involvement in HIV-1 entry,[2, 3] stem cell recruitment,[4] and various cancer processes, including angiogenesis and metastasis.[5] CXCR4 is a peptidergic GPCR, and the proven druggability of the GPCR superfamily means that small molecule CXCR4 antagonists have emerged as a promising class of antiretroviral/stem cell mobilizing/anticancer drugs. A number of peptide and non-peptide antagonists for CXCR4 has been reported,[4, 6, 7] but the haematopoietic stem cell mobilizing agent plerixafor[8] remains the only marketed drug in this class. Development of novel, drug-like CXCR4 antagonists has been further encouraged by the recently published X-ray structure of CXCR4.[9] In our continuing efforts towards peptidomimetic CXCR4 antagonists, we have used the peptide antagonists developed by Fujii and co-workers[10] as starting point. In a successful attempt to downsize the 14-mer peptide antagonist T140, Fujii's group discovered the potent cyclopentapeptide antagonist cyclo-(-Arg[1]-Arg[2]-2-Nal[3]-Gly[4]-D-Tyr[5]-),[11] today known as FC131 (**1**) (Fig. 1A). Several groups, including our own, have attempted to identify the binding mode of **1** based on molecular modeling studies, i.e. docking of cyclopentapeptide ligands to a receptor model.[12-16] However, in the absence of an experimentally determined binding mode for **1**, rational development of peptidomimetic analogs still relies heavily on the information obtained from active and inactive cyclopentapeptides in SAR studies, i.e. ligand-based design. To maximize the informational content of ligand-based design, ligands with limited conformational freedom are required, i.e. compounds that adopt a limited number of low-energy conformations. Despite its small size, the cyclopentapeptide backbone is still a relatively flexible template that can adopt a number of low-energy conformations.[17] For the cyclopentapeptide CXCR4 antagonists, this picture is further complicated by the presence of two highly flexible arginine side chains. On this background, we set out to develop conformationally constrained cyclopentapeptides to further probe the small peptide binding pocket of CXCR4. Here we report the successful application of two different rigidification strategies, along with a rationalization of the obtained SAR data. Additionally, we propose a binding mode that is consistent with the observed SAR.

RESULTS AND DISCUSSION

Chemistry. The cyclopentapeptide target compounds **1-16** (Fig. 1) were prepared by a combination of solid- and solution-phase synthesis as depicted in Scheme 1. Synthesis of the linear pentapeptide precursors was carried out by standard Fmoc-based solid-phase peptide synthesis using a trityl resin pre-loaded with Fmoc-Gly; Gly was chosen as the C-terminal residue to avoid epimerization in the subsequent cyclization step. The side chain protected peptide was selectively cleaved from the resin with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in DCM.[18] Head-to-tail cyclization was achieved in dilute solution (DMF-DCM 1:1) using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and *N*,*N*-diisopropylethylamine (DIPEA). Subsequent side chain deprotection, purification by preparative HPLC, and lyophilization gave the final products **1-8** and **10-16**. Compound **9** was obtained by guanidinylation of **8** with 1*H*-pyrazole-carboxamidine hydrochloride and DIPEA in DMF.

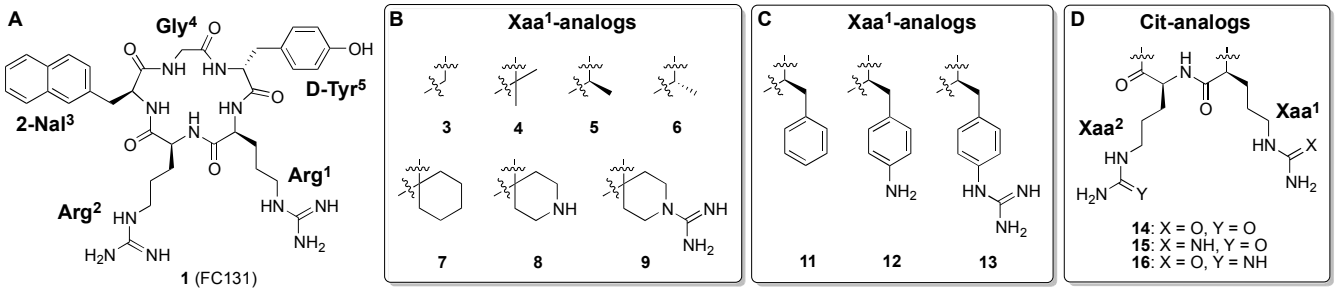
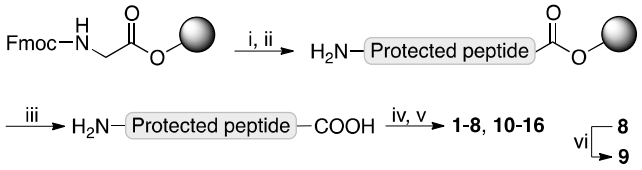


Figure 1. Structures of (A) the lead peptide **1**; (B) the backbone stabilizing Xaa[1]-analogs **3**-**9**; (C) the side chain rigidified Xaa[1]-analogs **11**-**13**; and (D) the citrulline analogs **14**-**16**.

Biology. The antagonistic potency of compounds **1-16** (Table 1) was determined in a functional assay measuring inhibition of CXCL12-induced activation of human CXCR4 transiently expressed in COS-7 cells. Briefly, the COS-7 cells were transfected with CXCR4 cDNA and the promiscuous G protein $G\alpha_{\Delta 6qi4myr}$, which turns the endogenous $G\alpha_i$-signal of CXCR4 into a $G\alpha_q$-signal that is easier to measure.[19] This method has been successfully applied in previous studies of CXCR4[20, 21] as well as of other chemokine receptors.[22, 23] Compounds **1-16** were tested in a concentration range from 10^{-8} to 10^{-4} M. See Supporting Information for experimental details.

Scheme 1. Synthetic strategy for target compounds 1-16[a]



[a]Reagents: (i) 20% piperidine in DMF; (ii) Fmoc-Xaa-OH, HBTU, DIPEA; (iii) HFIP-DCM (3:7); (iv) PyBOP, DIPEA; (v) TFA-TIS-H_2O (95:2.5:2.5); (vi) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA.

Design and SAR. The extracellular side of CXCR4 is highly negatively charged,[9] and the presence of arginine residues in positions 1 and 2 of the lead peptide **1** strongly suggests that molecular recognition is dominated by ionic interactions between the positively charged ligand and one or more negatively charged receptor residues. Previous SAR studies on Arg-/Lys-analogs of **1** have shown that a large variety of structural modifications are accepted in position 1,[24] while position 2 is very sensitive towards the same substitutions.[25] Thus, position 1 emerged as the ideal candidate for further structural tuning.

Backbone Stabilization. In order to arrive at conformationally constrained cyclopentapeptide CXCR4 antagonists with high potency, we were looking to include structural elements that would stabilize the bioactive backbone conformation of **1**. We have previously reported a 3D pharmacophore model that describes a specific all-*trans* conformation for the cyclopentapeptide backbone.[26] This computationally derived backbone conformation is consistent with the solution structure of **1**[11] and the conformational behavior of *N*-alkylated analogs, as determined by NMR.[16, 24, 27] For position 1 the backbone torsional angles (φ,ψ) are (-68˚, -48˚), which corresponds to the α_R-region of the Ramachandran plot. This "helical" backbone conformation is known to be stabilized by α,α-disubstituted α-amino acids.[28] Moreover, previous SAR studies have shown that both L- and D-Arg are accepted in position 1 (L-configuration slightly favored over D-configuration),[11] which we also confirmed (**1**, EC_{50} = 0.47 μM; [D-Arg[1]]FC131 (**2**), EC_{50} = 0.78 μM, Table 1). Taken together, these observations led us to investigate the stepwise introduction of an achiral α,α-disubstituted Arg-mimetic in position 1 (**3-9**, Fig. 1B, Table 1).

Table 1. Antagonistic potency of 1-16 on human CXCR4.

compd		log EC_{50} ± SEM[a]	EC_{50} (μM)
1[b]	FC131	-6.33 ± 0.04	0.47
2[b]	[D-Arg[1]]FC131	-6.11 ± 0.10	0.78
3	[Gly[1]]FC131	-4.44 ± 0.10	36
4	[Aib[1]]FC131	-5.45 ± 0.15	3.6
5[b]	[Ala[1]]FC131	-4.73 ± 0.21	19
6[b]	[D-Ala[1]]FC131	-4.71 ± 0.10	19
7	[Chx[1]]FC131	-5.27 ± 0.15	5.4
8	[Api[1]]FC131	-6.25 ± 0.14	0.56
9	[Gpi[1]]FC131	-6.59 ± 0.10	0.26
10[b]	[Orn[1]]FC131	-6.18 ± 0.20	0.66
11	[Phe[1]]FC131	-5.14 ± 0.04	7.2
12	[Aph[1]]FC131	-4.83 ± 0.10	15
13	[Gph[1]]FC131	-6.17 ± 0.16	0.68
14	[Cit[1,2]]FC131	>-4	>100
15	[Cit[2]]FC131	>-4	>100
16	[Cit[1]]FC131	-5.54 ± 0.14	2.9

[a]Values represent the mean of at least four independent experiments performed in duplicates. [b]Known compounds (the difference in potency relative to previous studies is likely due to differences in cellular background, receptor expression pattern and/or choice of functional readout).

Here, compound **3** ([Gly[1]]FC131) represents the baseline, i.e. maximal conformational flexibility and no side chain to provide receptor interactions. This modification resulted in a 77-fold reduction of the antagonistic potency (EC_{50} = 36 μM) relative to the reference compound **1**. We then introduced 2-aminoisobutyric acid (Aib), which is the simplest α,α-disubstituted α-amino acid. The resulting compound **4** ([Aib[1]]FC131) turned out to be 10-fold more potent (EC_{50} = 3.6 μM) than **3**, yet less potent than **1**. The achiral Aib can be considered a chimeric amino acid that combines the steric and conformational properties of L- and D-Ala. Indeed, the known compounds **5** and **6** ([Ala[1]]FC131 and [D-Ala[1]]FC131) were 5-fold less potent (EC_{50} = 19 μM for both compounds) than **4**. Since the two α-methyl groups of Aib are unlikely to provide

significant receptor interactions, the increased potency of **4** must mainly result from conformational effects, i.e. a stabilization of the bioactive backbone conformation. Thus, the beneficial effect of introducing Aib in position 1 supports the predicted backbone conformation of our independently generated 3D pharmacophore model.[26] Extension of Aib to the cyclohexane derivative (**7**, [Chx^1]FC131), containing additional hydrophobic bulk, resulted in a 1.5-fold reduction in potency (EC_{50} = 5.4 μM) compared to **4**. This could be expected since the most potent cyclopentapeptides are known to contain highly polar side chain functionalities in this position. Seeking to restore a favorable receptor interaction, we prepared the corresponding piperidine derivative **8** ([Api^1]FC131), which will be protonated at physiological pH. This resulted in a 10-fold increase in potency (EC_{50} = 0.56 μM) relative to **7**, which can be attributed to the positively charged side chain functionality. To come full circle, we prepared the corresponding guanidino-derivative **9** ([Gpi^1]FC131), which resulted in an additional 2-fold increase in potency (EC_{50} = 0.26 μM) relative to **8**. The effect of going from an amino- to a guanidino-derivative (**8** to **9**) is similar to what is seen for **1** (EC_{50} = 0.47 μM) relative to the corresponding amine **10** ([Orn^1]FC131, EC_{50} = 0.66 μM). Thus, for position 1 the delocalized charge and/or H-bond pattern of the guanidino group is slightly more favorable for the antagonistic potency.

Side Chain Rigidification. Obviously, the positioning of side chain functional groups relative to the backbone will depend on the number and values of side chain torsion angles (χ^n). For compounds **11**-**13** (Fig. 1C, Table 1), the conformational constraint was therefore introduced in the side chain. We chose to build the χ-restricted Arg-mimetics from Phe based on SAR data showing that Arg^1 can be substituted with 4-F-Phe without a dramatic loss of activity.[25] The potency of **11** ([Phe^1]FC131, EC_{50} = 7.2 μM) was similar to that of **7**, which again illustrates that non-polar side chain functionalities are unfavorable in position 1. Interestingly, introduction of a 4-amino substituent (**12**, [Aph^1]FC131) resulted in a further 2-fold reduction of potency (EC_{50} = 15 μM). However, the corresponding 4-guanidino derivative **13** ([Gph^1]FC131, EC_{50} = 0.68 μM) was almost equipotent with **1**, i.e. a 22-fold increased potency compared to **12**. These findings can be rationalized in terms of charge; at physiological pH the 4-amino group of **12** (aniline-type nitrogen, pK_a 4-5) will be uncharged,[29] while the 4-guanidino group of **13** (pK_a ~11) will be protonated.

Importance of Charge. To further investigate the importance of charge and the relative contribution from the two guanidino groups in **1**, we exchanged the arginines with citrulline (Cit), which is uncharged but partly retains the H-bonding pattern of Arg (Fig. 1D, Table 1). The double urea-analog **14** ([Cit^{1,2}]FC131) failed to show any antagonistic activity (EC_{50} > 100 μM). We then re-introduced the guanidino group in position 1 (**15**, [Cit^2]FC131); however, this was not sufficient to restore activity (EC_{50} > 100 μM). In contrast, re-introduction of the guanidino group in position 2 (**16**, [Cit^1]FC131) resulted in a moderately active compound (EC_{50} = 2.9 μM). These findings clearly demonstrate that the positively charged guanidino group in position 2 is crucial for activity, while the interaction provided by Arg^1 plays a secondary role.

Binding Mode. The compounds in the present study were designed to complement existing SAR data for positions 1 and 2 in cyclopentapeptide CXCR4 antagonists. The collective SAR data shows that Arg^2 is essential and must provide the anchor point for binding to CXCR4. For position 1, the only clear trend is that a positively ionizable group is preferred, which indicates the involvement of a salt bridge with a negatively charged receptor site. However, in contrast to position 2, the H-bond potential (guanidino or amino), configuration (L- or D-), and spacer properties (length, degree of flexibility/rigidity) are not critical. In an attempt to rationalize this data, **9** was docked to the X-ray structure of CXCR4 (bound to the 16-mer peptide antagonist CVX15; PDB code 3OE0)[9] using the induced fit method developed by Schrödinger.[30] Optimization of the docking protocol (see Supporting Information) resulted in the identification of a binding mode that is consistent with the observed SAR for positions 1 and 2 (Fig. 2).

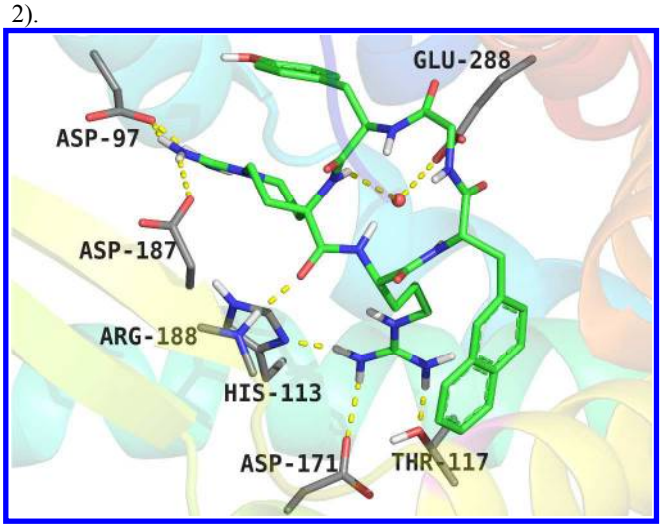


Figure 2. Suggested binding mode for **9**. The ligand is shown with green carbons, and receptor residues with grey carbons. Non-polar hydrogen atoms are omitted for clarity. H-bonds to the Gpi^1-Arg^2 fragment are shown as dotted yellow lines.

The Arg^2 side chain of **9** protrudes into a tight pocket, where the guanidino group is involved in a complex, charge-assisted H-bond network that involves several receptor residues (His113, Thr117, and Asp171). This is consistent with the strong preference for L-Arg (over similar Cit-/Arg-/Lys-analogs) in position 2, i.e. a simultaneous importance of charge, H-bond potential, configuration, and spacer length. The guanidino group of the Gpi^1 side chain establishes ionic interactions with both Asp97 and Asp187; thus, the Gpi^1-Arg^2 fragment of **9** binds in a similar fashion as the Arg^1-Arg^2 fragment of CVX15.[9] The backbone NH of Gpi^1 is involved in a water-mediated H-bond to Glu288, while its backbone CO forms a H-bond with the backbone NH of Arg188. These backbone interactions would explain the importance of maintaining the correct backbone conformation around position 1, as demonstrated in the present paper.

Docking of **1** resulted in a similar pose, where its Arg^1 side chain binds to Asp97 and Asp187 in the same way as the Gpi^1 side chain of **9** (results not shown). However, closer inspection of the binding region for Xaa^1 revealed that it is relatively open and contains several potential binding partners for a positively ionizable group. Indeed, docking of **13** showed that its longer 4-guanidino-Phe^1 side chain binds to Glu288 instead of Asp97/Asp187 (results not shown). Thus, in order to explain the relatively "flat" SAR for position 1, it can be envisaged

that the different Xaa[1] side chains bind to different receptor subsites within this region.

Even if the χ-angles were allowed to rotate during docking, the side chain orientations of the receptor-bound **9** are generally consistent with our 3D pharmacophore model.[26] However, the binding mode presented here is very different from our previously reported pose, where Glu288 was proposed to be the anchor point for Arg[2].[12] That pose was generated by docking cyclopentapeptide ligands to a homology model based on the rhodopsin structure, which today is considered as a suboptimal template for CXCR4. Consequently, the present binding mode has more in common with poses from recent docking studies based on the X-ray structure of CXCR4. It is virtually identical with the pose suggested for **1** by Yoshikawa et al.,[15] and except for the rotameric state of D-Tyr[5], it is also similar to the binding mode for **10** suggested by Demmer et al.[14] However, Demmer et al. recently proposed an "inverted" binding mode for a potent *N*-alkylated cyclopentapeptide derivative, which involved Asp97 as the anchor point for Arg[2].[16] Clearly, the binding mode for cyclopentapeptide CXCR4 antagonists still needs to be determined experimentally, and we have recently initiated extensive site-directed mutagenesis studies towards this end.

To conclude, we have rationally designed two different types of conformationally constrained cyclopentapeptide CXCR4 antagonists that shed further light on the structural requirements for small peptide binding to CXCR4. Importantly, introduction of an α,α-disubstituted amino acid in position 1 (i.e. compounds **4** and **9**) is beneficial for activity, which supports the predicted bioactive backbone conformation for this compound class.

EXPERIMENTAL SECTION

Chemistry. General. All reagents and solvents were purchased and used as received without further purification. UHPLC/HPLC and MS were used for routine analysis of crude products and monitoring of reactions in solution, and all end products were purified by preparative HPLC. Different gradients of CH_3CN-H_2O, containing 0.1% (*v*/*v*) TFA, were used as eluting solvent in all chromatography systems, with photodiode array detection at 214 or 254 nm. The UHPLC system consisted of a Waters ACQUITY UPLC H-Class equipped with a Waters ACQUITY UPLC BEH C18 reversed phase column (1 × 150 mm, 1.75 µm particle size, 0.120 mL/min flow rate). Analytical HPLC was performed on a Waters 2695 system equipped with an XBridge™ C18 reversed phase column (250 mm × 4.6 mm, 5 µm particle size, 1 mL/min flow rate). Preparative HPLC was performed on a Waters 600 Semi Prep System equipped with an XBridge™ C18 reversed phase preparative column (250 mm × 19 mm, 10 µm particle size, 15 mL/min flow rate). Routine MS was performed using a Waters MALDI micro MX instrument, and HRMS spectra of the end products were obtained on an LTQ Orbitrap XL (Thermo Scientific, Bremen, Germany) using positive-mode ESI. ^{1}H NMR spectra were acquired on a 400 MHz Varian spectrometer; chemical shifts are expressed in ppm relative to solvent (CD_3OD or DMSO-d_6) signals. All end products were >95% pure as determined by analytical HPLC.

General Procedure for Synthesis of Cyclic Pentapeptides (1-8, 10-16). The linear pentapeptide precursors were synthesized manually by standard Fmoc-based solid-phase peptide synthesis, starting from pre-loaded Fmoc-Gly-NovaSyn TGT® resin (0.20 mmol/g). All amino acid building blocks were N^{α}-Fmoc-protected, and the following side chain protecting groups were used: pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) for Arg; *t*-Bu for D-Tyr; Boc for Orn, Aph, Gph, and Api. Briefly, the resin (1 g, 0.20 mmol) was swollen with DCM and washed with DMF. Before each coupling step, the Fmoc protecting group was removed with 20% piperidine in DMF (5 min × 3). Amino acid coupling was performed by adding a preactivated solution containing 4 equiv each of Fmoc-protected amino acid, *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU), and DIPEA in DMF, and allowing to react for 1 h. For compounds **7** and **8**, double coupling of D-Tyr[5] was performed due to steric hindrance of the preceding residue. Selective cleavage of the side chain protected peptide was accomplished by treating the resin with 5 mL HFIP-DCM (3:7) (*v*/*v*) (5 min × 3), followed by removal of solvent *in vacuo*. The crude linear peptide was dissolved in DCM-DMF (1:1) (*v*/*v*) to a concentration of 0.5 mg/mL, and cyclized overnight with 2 equiv each of PyBOP and DIPEA. The solvent was removed *in vacuo*, and the crude cyclic product was deprotected by adding 15 mL TFA-TIS-H_2O (95:2.5:2.5) (*v*/*v*) and allowing to react for a minimum of 2 h. The crude end product was precipitated by cold diethyl ether, purified by preparative HPLC, and lyophilized.

Synthesis of cyclo-(-Gpi[1]-Arg[2]-2-Nal[3]-Gly[4]-D-Tyr[5]-) (9). Compound **8** (28 mg, 0.03 mmol) was dissolved in DMF (25 mL) and 5 equiv each of 1*H*-pyrazole-1-carboxamidine hydrochloride (22 mg, 0.15 mmol) and DIPEA (26 µL, 0.15 mmol) were added. After 24 h, the starting material was still present, so another 5 equiv of each reagent were added, and the reaction stirred until starting material could not longer be observed. Concentration under reduced pressure, purification by preparative HPLC, and lyophilization gave **9** (13 mg, 44% from **8**) as a white powder.

ASSOCIATED CONTENT

Supporting Information

Yields and characterization data (HRMS, purity, ^{1}H NMR) for **1-16**, experimental procedure for biological assay, computational procedure for docking. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +47 77 62 09 09; e-mail: jon.vabeno@uit.no.

Present Address

[§]Northern Research Institute Tromsø, P.O. Box 6434, Tromsø Science Park, NO-9294 Tromsø, Norway.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Financial support for this project was obtained from the Research Council of Norway (grant 190728/V30) (J.M. and J.V.), and from the University of Copenhagen and the Danish Medical Research Council (S.T. and M.M.R). We thank Zack G. Zachariassen for helping to establish the protocol for manual peptide synthesis, and Hawi G. Daba for assisting in the docking studies. We would also like to thank Inger S. Simonsen and Randi Thøgersen for invaluable technical assistance in the biological assay.

ABBREVIATIONS

2-Nal, L-3-(2-naphthyl)-alanine; Aib, 2-aminoisobutyric acid; Aph, L-4-amino-phenylalanine; Api, 4-aminopiperidine-4-carboxylic acid; Chx, 1-aminocyclohexane-1-carboxylic acid; Cit, L-citrulline; CXCL12, chemokine (C-X-C motif) ligand 12; CXCR4, C-X-C chemokine receptor 4; DIPEA, *N*,*N*-diisopropylethylamine; Gph, L-4-guanidino-phenylalanine; Gpi, 4-amino-1-carbamimidoylpiperidine-4-carboxylic acid; HBTU, *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; Orn, L-ornithine; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TIS, triisopropylsilane; Xaa, any amino acid.

REFERENCES

(1) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. International Union of Pharmacology. XXII. Nomenclature for Chemokine Receptors. *Pharmacol. Rev.* **2000,** 52, 145-176.

(2) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor. *Science* **1996,** 272, 872-877.

(3) Berson, J. F.; Long, D.; Doranz, B. J.; Rucker, J.; Jirik, F. R.; Doms, R. W. A Seven-Transmembrane Domain Receptor Involved in Fusion and Entry of T-Cell-Tropic Human Immunodeficiency Virus Type 1 Strains. *J. Virol.* **1996,** 70, 6288-6295.

(4) Steen, A.; Rosenkilde, M. M. Molecular Pharmacology of CXCR4 Inhibition. In *Novel Developments in Stem Cell Mobilization: Focus on CXCR4*, Fruehauf, S.; Zeller, W. J.; Calandra, G., Eds. Springer: 2012; pp 23-37.

(5) Balkwill, F. The Significance of Cancer Cell Expression of the Chemokine Receptor CXCR4. *Semin. Cancer Biol.* **2004,** 14, 171-179.

(6) Singh, I. P.; Chauthe, S. K. Small Molecule HIV Entry Inhibitors: Part I. Chemokine Receptor Antagonists: 2004 - 2010. *Expert Opin. Ther. Patents* **2011,** 21, 227-269.

(7) Choi, W. T.; Duggineni, S.; Xu, Y.; Huang, Z.; An, J. Drug Discovery Research Targeting the CXC Chemokine Receptor 4 (CXCR4). *J. Med. Chem.* **2012,** 55, 977-994.

(8) DiPersio, J. F.; Uy, G. L.; Yasothan, U.; Kirkpatrick, P. Plerixafor. *Nat. Rev. Drug Discov.* **2009,** 8, 105-107.

(9) Wu, B.; Chien, E. Y. T.; Mol, C. D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F. C.; Hamel, D. J.; Kuhn, P.; Handel, T. M.; Cherezov, V.; Stevens, R. C. Structures of the CXCR4 Chemokine GPCR with Small-Molecule and Cyclic Peptide Antagonists. *Science* **2010,** 330, 1066-1071.

(10) Oishi, S.; Fujii, N. Peptide and Peptidomimetic Ligands for CXC Chemokine Receptor 4 (CXCR4). *Org. Biomol. Chem.* **2012**.

(11) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z. X.; Peiper, S. C. Molecular-Size Reduction of a Potent CXCR4-Chemokine Antagonist Using Orthogonal Combination of Conformation- and Sequence-Based Libraries. *Angew. Chem. Int. Ed. Engl.* **2003,** 42, 3251-3253.

(12) Våbenø, J.; Nikiforovich, G. V.; Marshall, G. R. Insight into the Binding Mode for Cyclopentapeptide Antagonists of the CXCR4 Receptor. *Chem. Biol. Drug Des.* **2006,** 67, 346-354.

(13) Kawatkar, S. P.; Yan, M.; Gevariya, H.; Lim, M. Y.; Eisold, S.; Zhu, X.; Huang, Z.; An, J. Computational Analysis of the Structural Mechanism of Inhibition of Chemokine Receptor CXCR4 by Small Molecule Antagonists. *Exp. Biol. Med.* **2011,** 236, 844-850.

(14) Demmer, O.; Dijkgraaf, I.; Schumacher, U.; Marinelli, L.; Cosconati, S.; Gourni, E.; Wester, H. J.; Kessler, H. Design, Synthesis, and Functionalization of Dimeric Peptides Targeting Chemokine Receptor CXCR4. *J. Med. Chem.* **2011,** 54, 7648-7662.

(15) Yoshikawa, Y.; Kobayashi, K.; Oishi, S.; Fujii, N.; Furuya, T. Molecular Modeling Study of Cyclic Pentapeptide CXCR4 Antagonists: New Insight into CXCR4-FC131 Interactions. *Bioorg. Med. Chem. Lett.* **2012,** 22, 2146-2150.

(16) Demmer, O.; Frank, A. O.; Hagn, F.; Schottelius, M.; Marinelli, L.; Cosconati, S.; Brack-Werner, R.; Kremb, S.; Wester, H.-J.; Kessler, H. A Conformationally Frozen Peptoid Boosts CXCR4 Affinity and Anti-HIV Activity. *Angew. Chem. Int. Ed. Engl.* **2012,** 51, 8110-8113.

(17) Nikiforovich, G. V.; Kövér, K. E.; Zhang, W.-J.; Marshall, G. R. Cyclopentapeptides as Flexible Conformational Templates. *J. Am. Chem. Soc.* **2000,** 122, 3262-3273.

(18) Bollhagen, R.; Schmiedberger, M.; Barlos, K.; Grell, E. A New Reagent for the Cleavage of Fully Protected Peptides Synthesised on 2-Chlorotrityl Chloride Resin. *J. Chem. Soc., Chem. Commun.* **1994**, 2559 - 2560.

(19) Kostenis, E.; Zeng, F. Y.; Wess, J. Functional Characterization of a Series of Mutant G Protein α_q Subunits Displaying Promiscuous Receptor Coupling Properties. *J. Biol. Chem.* **1998,** 273, 17886-17892.

(20) Rosenkilde, M. M.; Gerlach, L.-O.; Jakobsen, J. S.; Skerlj, R. T.; Bridger, G. J.; Schwartz, T. W. Molecular Mechanism of AMD3100 Antagonism in the CXCR4 Receptor: Transfer of Binding Site to the CXCR3 Receptor. *J. Biol. Chem.* **2004,** 279, 3033-3041.

(21) Rosenkilde, M. M.; Gerlach, L.-O.; Hatse, S.; Skerlj, R. T.; Schols, D.; Bridger, G. J.; Schwartz, T. W. Molecular Mechanism of Action of Monocyclam *Versus* Bicyclam Non-Peptide Antagonists in the CXCR4 Chemokine Receptor. *J. Biol. Chem.* **2007,** 282, 27354-27365.

(22) Jensen, P. C.; Thiele, S.; Ulven, T.; Schwartz, T. W.; Rosenkilde, M. M. Positive *Versus* Negative Modulation of Different Endogenous Chemokines for CC-Chemokine Receptor 1 by Small Molecule Agonists through Allosteric *Versus* Orthosteric Binding. *J. Biol. Chem.* **2008,** 283, 23121-23128.

(23) Thiele, S.; Steen, A.; Jensen, P. C.; Mokrosinski, J.; Frimurer, T. M.; Rosenkilde, M. M. Allosteric and Orthosteric Sites in CC Chemokine Receptor (CCR5), a Chimeric Receptor Approach. *J. Biol. Chem.* **2011,** 286, 37543-37554.

(24) Tamamura, H.; Araki, T.; Ueda, S.; Wang, Z.; Oishi, S.; Esaka, A.; Trent, J. O.; Nakashima, H.; Yamamoto, N.; Peiper, S. C.; Otaka, A.; Fujii, N. Identification of Novel Low Molecular Weight CXCR4 Antagonists by Structural Tuning of Cyclic Tetrapeptide Scaffolds. *J. Med. Chem.* **2005,** 48, 3280-3289.

(25) Tamamura, H.; Esaka, A.; Ogawa, T.; Araki, T.; Ueda, S.; Wang, Z.; Trent, J. O.; Tsutsumi, H.; Masuno, H.; Nakashima, H.; Yamamoto, N.; Peiper, S. C.; Otaka, A.; Fujii, N. Structure-Activity Relationship Studies on CXCR4 Antagonists Having Cyclic Pentapeptide Scaffolds. *Org. Biomol. Chem.* **2005,** 3, 4392-4394.

(26) Våbenø, J.; Nikiforovich, G. V.; Marshall, G. R. A Minimalistic 3D Pharmacophore Model for Cyclopentapeptide CXCR4 Antagonists. *Biopolymers (Pept. Sci.)* **2006,** 84, 459-471.

(27) Ueda, S.; Oishi, S.; Wang, Z. X.; Araki, T.; Tamamura, H.; Cluzeau, J.; Ohno, H.; Kusano, S.; Nakashima, H.; Trent, J. O.; Peiper, S. C.; Fujii, N. Structure-Activity Relationships of Cyclic Peptide-Based Chemokine Receptor CXCR4 Antagonists: Disclosing the Importance of Side-Chain and Backbone Functionalities. *J. Med. Chem.* **2007,** 50, 192-198.

(28) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; di Blasio, B.; Pavone, V.; Pedone, C. Preferred Conformations of Peptides Containing α,α-Disubstituted α-Amino Acids. *Biopolymers* **1983,** 22, 205-215.

(29) Sun, N.; Avdeef, A. Biorelevant pK_a (37 °C) Predicted from the 2D Structure of the Molecule and Its pK_a at 25 °C. *J. Pharm. Biomed. Anal.* **2011,** 56, 173-182.

(30) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects. *J. Med. Chem.* **2006,** 49, 534-553.

Table of Contents graphic