Design and synthesis of all diastereomers of cyclic pseudo-dipeptides as mimics of cyclic CXCR4 pentapeptide antagonists†

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The four diastereomers of 2,5-bis[(3-guanidino)propyl]-1-[3-(4-hydroxyphenyl)propionyl]-7-(2-naphthylacetyl)-1,4,7-triazacycloundec-9-en-3-one (**54**–**57**) and of 2,5-bis[(3-guanidino)propyl]-1-(4-hydroxyphenylacetyl)-7-(2-naphthylacetyl)-1,4,7-triazacycloundec-9-en-3-one (**58**–**61**) were synthesized by a divergent methodology from L- and D-glutamic acids. The 11-membered ring core was made by ring closing metathesis of linear bis(allylamines), and the guanidyl functions were introduced by a simultaneous double Mitsunobu reaction using bis(Boc)guanidine. These compounds were designed to mimic cyclic pentapeptide FC131 (c[Gly-D-Tyr-Arg-Arg-Nal]).

Introduction

CXCR4 chemokine receptor is involved in HIV-1 infection of T cells. Attachment of virus envelope glycoprotein gp120 onto cell surface proteins CD4 and CXCR4 leads to membrane fusion and subsequent virus entry into the cell.[1,2] Thus, CXCR4 is considered an important therapeutic target. Several potent CXCR4 antagonists have been developed so far. Among these, we have previously identified a β-sheet-like 14-residue peptide[3] T140, and its down-sized analogues, cyclic pentapeptide FC131[4] (c[Gly-D-Tyr-Arg-Arg-Nal]) as potent and specific CXCR4 antagonists. These were characterized as HIV-1 entry inhibitors (Fig. 1).[5] Several other non-peptidic, low-molecular-weight CXCR4 antagonists have also been reported to inhibit HIV-1 infection through CXCR4, such as KRH2731[6] and AMD3100.[7] AMD3100 was abandoned as an anti-HIV drug[8] because of a lack of *in vivo* efficacy and undesirable side effects; nevertheless, AMD070, an orally-available derivative of AMD3100 has recently been described to be as potent as AMD3100, and it will be further investigated as an HIV drug in clinical trials.[9,10]

The use of constrained peptides or peptide mimics has become popular for increasing receptor affinity, for the development of new drugs, to investigate bioactive conformations, and to increase duration of action. On native peptides, constraints can be introduced by linkage between two points of the peptide (NH to NH,[11] NH to CO_2H[12] or disulfide bridge[13]) to fix a large secondary structure, generally a helix or sheets. The small secondary structures (turn, small helix, hairpin and *E*- or *Z*-amide bonds) can also be induced, stabilized or fixed by introduction in the sequence of one or several small constrained (Freidinger lactams[14] or azabicycloalkanes[15,16]), bulky (alkylprolines[17]) or rigid (dipeptide isosteres[18,19]) unnatural amino acids. Finally, small peptides or parts of peptides can be converted to small semi-peptidic or peptide-like structures, synthesized to mimic peptide backbones, and possessing all the necessary functional groups for activity.[20]

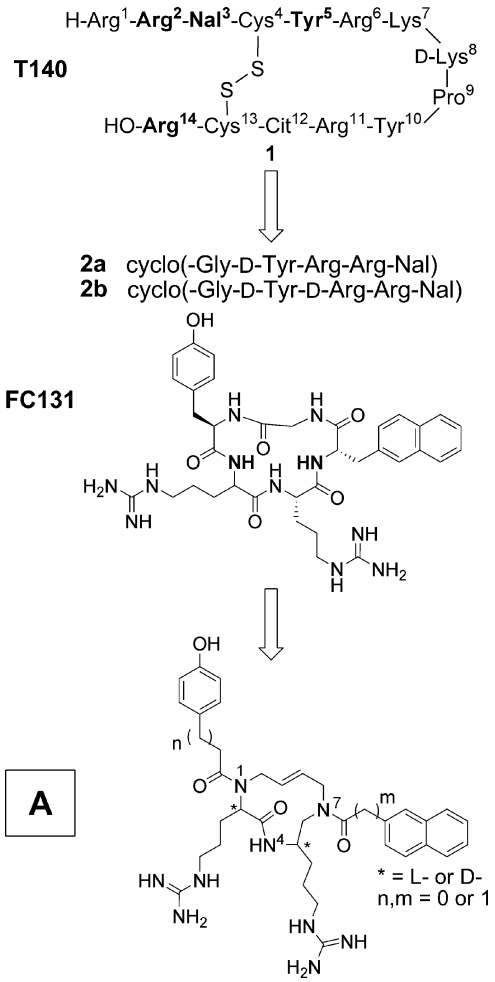

Fig. 1 Down-sizing CXCR4 antagonists.

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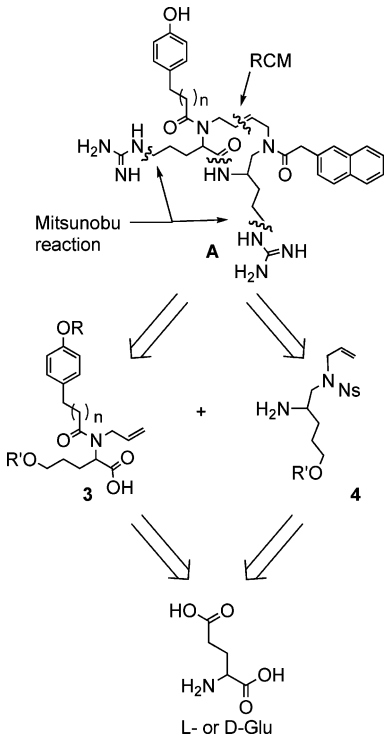
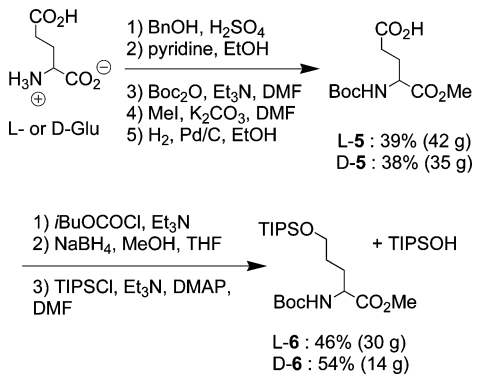
In our research on the development of new CXCR4 receptor antagonists, we are looking for compounds with a lower molecular weight than our current active peptide FC131, and with a more stable and active structure. Our previous studies on FC131 led us to modify its sequence and to use a linkage between two points of the peptide by replacing Gly[1] by an alkyl chain or disulfide bridge.[13a] Unfortunately these modifications resulted in significant loss of activity. Unnatural amino acids were also introduced to the peptide sequence to perform structure–activity relationship studies on the peptide backbone. For example, the replacement of Arg[4] by constrained amino acids *cis*- or *trans*-4-guanidino-Pro furnished peptides with similar activities to that of the parent peptide, showing the importance of the constrained structure of the peptide backbone.[13a] On the other hand, introduction of *E*-alkene dipeptide isosteres in positions 4–5 (Arg–Nal) and 5–1 (Nal–Gly) led to peptides with similar pharmacophore orientations and distances, and revealed the importance of amide bonds on the backbone of the peptide.[19] We are also interested in the synthesis of small semi-peptidic compounds containing pharmacophores of FC131. We have recently reported that a 3,6-dihydropyridin-2-one analog containing two guanidine residues and a naphthyl group retains moderate activity as a CXCR4 antagonist.[21] We are now interested in a less-rigid backbone structure, on which it would be easy to vary side-chain length and chirality. Design of compounds of type **A** was performed by removing Gly from the parent peptide and making a linkage between the nitrogens (bold) of Arg and Nal (Fig. 1). Such a structure could possess a propionyl or acetyl (*n*, $m = 0$ or 1) side chain to replace tyrosine and naphthylalanine, and propenyl linkage between nitrogens 1 and 7 to constrain dipeptides and form 11-membered ring compounds.

Retrosynthetically, the ring can be closed by ring closing metathesis (RCM) between two allylamine residues. RCM has been shown to be a useful method for synthesizing constrained peptides and forming 8–10-membered rings[20a,22] and 13–20-membered ring cycles.[11,12,20b,c,d] Nevertheless, the only reported example of RCM on an 11-membered, ring-constrained peptide failed,[11] but we expected that cyclization would be possible with a new generation of catalysts. Cyclization of these peptides was reported to be possible without protection of the amide bond to increase *cis* conformation for cycles of up to 10-membered rings. To simplify the synthesis, the two nucleophilic guanidyl groups should be introduced in the last steps by the Mitsunobu reaction with corresponding alcohols (Scheme 1). Our two fragments were *N*-substituted 5-hydroxynorvaline acids **3** and diamines **4**. Both of these could be synthesized from the same precursor, L- or D-glutamic acid, to give easy access to the four possible diastereomers.



Scheme 1 Retrosynthesis of compounds **A**.



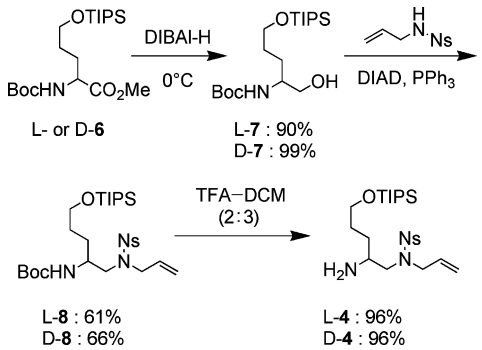
Scheme 2 Synthesis of protected L- and D-5-hydroxynorvaline.

Results and discussion

Synthesis of 5-hydroxynorvaline, also called pentahomoserine, has been reported many times in protected and unprotected forms.[23] We chose to use the procedure described by Kokotos[24] from glutamic acid (Scheme 2). L- and D-Glu were efficiently monobenzylated using benzyl alcohol, H_2SO_4 and pyridine;[25] the amine was protected with Boc; the α-acid was converted to a methyl ester, and the benzyl ester was removed by hydrogenation with Pd/C to give the free acids **5**, on a large scale. Reduction of acids to alcohols using the mixed anhydrides and protection with TIPSCl provided the fully protected L-5-hydroxynorvaline L-**6** at a yield of 46% (82% brsm), and the D-5-hydroxynorvaline D-**6** in a similar manner and yield. Both amino acids **6** were obtained as inseparable mixtures with TIPSOH, but this alcohol did not interfere with the next steps, and yields were estimated by comparing ^{1}H-NMR integration of TIPS and other signals.

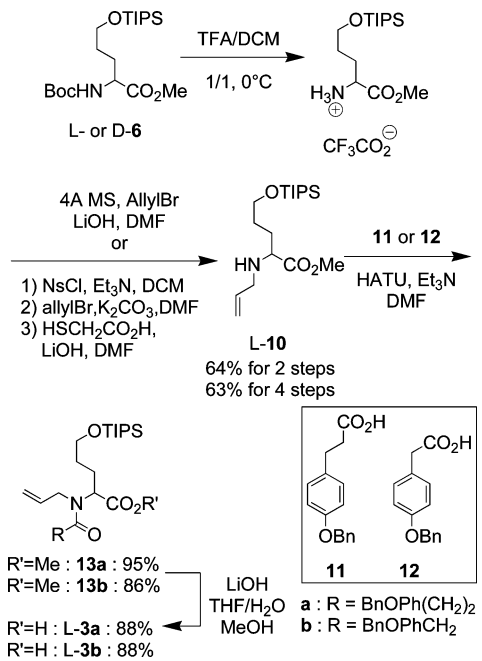
For the synthesis of diamines L- and D-**4**, esters **6** were reduced to alcohols **7** using DIBAl-H in THF (Scheme 3). Protected amines **8** were obtained by the Mitsunobu reaction between alcohols **7** and *ortho*-nitrobenzenesulfonyl allylamine (Ns-allylamine) **9**, with moderate yields. Boc removal to form both amines L- and D-**4** in TFA–DCM (2 : 3) were carried out just before coupling with acids **3**.

For the synthesis of acids **3**, conversion of Boc carbamate to allyl amine was carried out on a small scale with a two-step procedure. Boc removal with TFA and selective mono-allylation of the free amine using the Kim protocol gave allyl amine **10**.[26] Unfortunately, we were not able to obtain a good yield of monoallylamine on the



Scheme 3 Synthesis of amines **4**.

5 g scale (35%) by this method, and diallylamine became the major product. We decided to use a longer but more reliable method using an *ortho*-nitrobenzenesulfonyl (Ns) protecting group (Scheme 4). Boc carbamate was removed with TFA and replaced by a nosyl group by reaction with NsCl and Et_3N in DMF. Nosyl amine was easily allylated using allyl bromide and potassium carbonate in DMF, and the nosyl group was removed using mercaptoacetic acid under basic conditions to give **10**, with a 63% yield, from **6** on a 5 g scale. Allylamine **10** was then coupled with acids **11** and **12** using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[27] in DMF to give allylamides **13a** and **13b**, with 95 and 86% yields, respectively. Saponification of the methyl esters with LiOH furnished the desired acids L-**3a** and L-**3b**.[28]

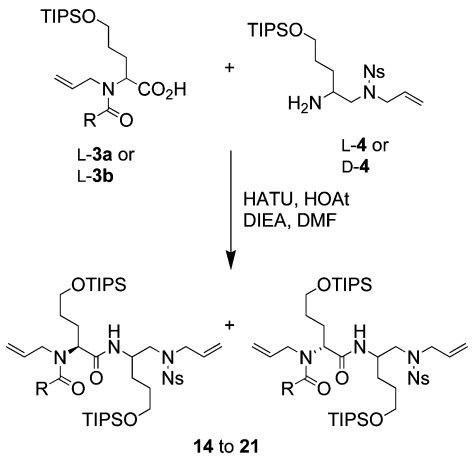


Scheme 4 Synthesis of acids **3a** and **3b**.

The amino acid coupling of acid L-**3a** and amine L-**4** proceeded completely using HATU, 1-hydroxy-7-azabenzotriazole (HOAt) and DIEA in DMF, but gave a 1 : 1 mixture of two inseparable diastereomers, **14** and **15** (Scheme 5, Table 1, entry 1). We were not able to suppress epimerization of the acid during the coupling step. We decided to use this epimerization to our advantage for the synthesis of the different diastereomers. Acid L-**3a** was coupled

Table 1 Amino acid coupling of compounds **3** and **4**

	Acid	Amine	R	Products (yield)
1	L-**3a**	L-**4**	$BnOPh(CH_2)_2$	**14**/**15** (88%)
2	L-**3a**	D-**4**	$BnOPh(CH_2)_2$	**16**/**17** (91%)
3	L-**3b**	L-**4**	$BnOPhCH_2$	**18** (43%) **19** (48%)
4	L-**3b**	D-**4**	$BnOPhCH_2$	**20** (37%) **21** (53%)



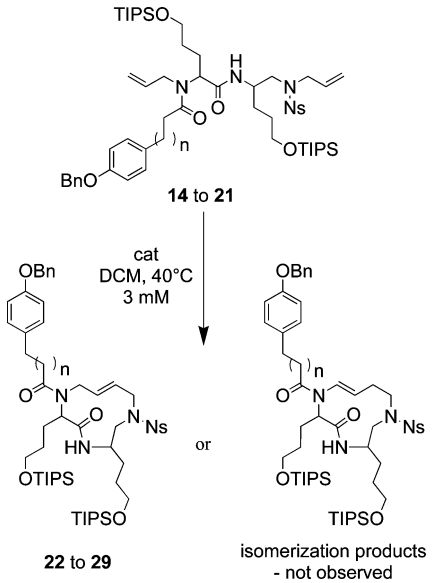
Scheme 5 Synthesis of dienes **14** to **21**.

with amine D-**4** using the same conditions and gave a mixture of **16** and **17**. Acid L-**3b** was coupled with amines L-**4** and D-**4** to give separable mixtures of diastereomers **18**/**19** and **20**/**21** in good yields.

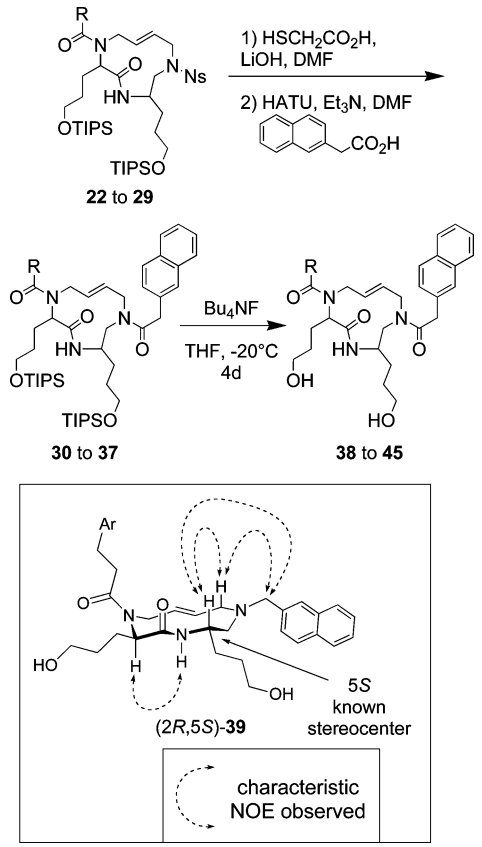
RCM of allylamines has been reported to react differently depending on the temperature and the catalyst used. Grubb's I and Grubb's II catalysts have been reported to lead to partial or total isomerization of the olefin, depending on reaction temperature and substrates, to give cyclic or acyclic enamides.[22e,29] This isomerization process does not seem to be general, indeed normal reactivity has also been reported on similar substrates with both catalysts.[30] We first attempted RCM on the diene mixture **14**/**15** using 0.2 eq. of Grubb's catalysts of first and second generation, in CH_2Cl_2 at reflux and high dilution (6 to 3 mM, Scheme 6). Reaction with Grubb's I catalyst proceeded slowly and was not completed within 24 h. A low yield of the desired separable 11-membered ring diastereomers **22** and **23** was nevertheless obtained (Table 2, entry 1). Surprisingly, in contrast to the reported observation of RCM with allylamines, reaction with Grubb's II catalyst was completed after 12 h and gave a good yield of diastereomers **22** and **23**. (Table 2, entry 2). The structures of **22** and **23** were confirmed using COSY NMR spectra of **22** and **39** (derived from **23**). Both

Table 2 RCM of linear dienes

	Diene	*n*	Grubb's catalyst	Product (yield)
1	**14**/**15**	1	I	**22** (13%) + **23** (25%)
2	**14**/**15**	1	II	**22** (43%) + **23** (42%)
3	**16**/**17**	1	II	**24** (38%) + **25** (35%)
4	**18**	0	II	**26** (69%)
5	**19**	0	II	**27** (65%)
6	**20**	0	II	**28** (71%)
7	**21**	0	II	**29** (67%)



Scheme 6 RCM of diallylamines **14**–**21** to 11-membered rings **22**–**29**.



Scheme 7 Introduction of naphthylacetyl side chain and removal of TIPS protecting groups. Expected structure of compound **39** using NOE correlation.

compounds had the desired but-2-ene-1,4-diamine structure, but enamide structures coming from olefin isomerization anticipated for the reaction with Grubb's II catalyst at high temperature were not observed.[31]

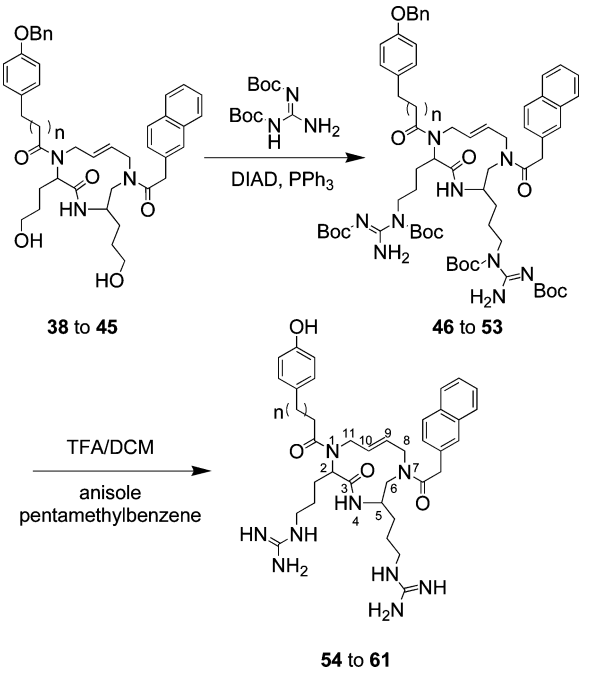
The diastereomeric mixture **16**/**17** and the single diastereomers **18**–**21** were then cyclized using Grubb's II catalyst in DCM at 40 °C, to give products **24**–**29** in good yields. All the compounds appeared to have *E*-olefins; *Z*-olefins were not observed.

Introduction of naphthylacetyl side chains was easily achieved by removal of nosyl groups using mercaptoacetic acid under basic conditions on cycles **22**–**29** to form the free amines, which were directly acylated with naphthylacetic acid using HATU and triethylamine, to give compounds **30**–**37** (Scheme 7, Table 3). These compounds were then submitted to tetrabutylammonium fluoride (TBAF) solution at −20 °C to remove both TIPS groups. The first TIPS group was completely removed in less than 24 h (by TLC), but removal of the second TIPS appeared to be very slow and needed a large excess of TBAF. Increasing the temperature up to −20 °C accelerated the rate of deprotection but led to formation of unidentified side products on TLC and coloration of the solution. After 72–96 h at −20 °C with 6 eq. TBAF, diols **38**–**45** were isolated in moderate to good yields. The structures and relative stereochemistry of diastereomers were established based on the known stereochemistry at carbon 5 and one- and two-dimensional NMR experiments. The majority of the NMR signals were well resolved at distinct chemical shifts for product **39**. All protons were assigned using the COSY experiment, which established their linear sequence around the macrocycle. A large coupling constant for NH proton (J_{NH} 9.5 Hz),[32] indicating a dihedral angle of −130° or −110°, and observation in the NOESY spectra of a correlation between C_2 proton and NH allowed us to attribute the (2*R*,5*S*) stereochemistry to compound **39**, and therefore to other diastereomers. Unfortunately, we were not able to get clearer proof of the stereochemistry at C_2.

Introduction of a guanidyl group *via* the Mitsunobu reaction can be achieved using bis- or tris-Boc- or Cbz-guanidine (Scheme 8).[23c,33] We decided to use the bis-Boc-guanidine for the acidic cleavage of the Boc group and because bis-Boc has only one reactive site while tris-Boc has the possibility to react twice. The reaction worked well with simple alcohols, with yields up to 90%,[33c] but gave average results on amino acids, with yields of 40–63%. In

Table 3 Introduction of naphthyl side chain and TIPS deprotection

	Ns amine	R	Acyl product (yield, 2 steps)	Diol (yield)
1	(2*S*,5*S*)-**22**	$BnOPh(CH_2)_2$	**30** (63%)	**38** (78%)
2	(2*R*,5*S*)-**23**	$BnOPh(CH_2)_2$	**31** (80%)	**39** (74%)
3	(2*R*,5*R*)-**24**	$BnOPh(CH_2)_2$	**32** (44%)	**40** (72%)
4	(2*S*,5*R*)-**25**	$BnOPh(CH_2)_2$	**33** (65%)	**41** (72%)
5	(2*S*,5*S*)-**26**	$BnOPhCH_2$	**34** (63%)	**42** (59%)
6	(2*R*,5*S*)-**27**	$BnOPhCH_2$	**35** (66%)	**43** (98%)
7	(2*R*,5*R*)-**28**	$BnOPhCH_2$	**36** (58%)	**44** (89%)
8	(2*S*,5*R*)-**29**	$BnOPhCH_2$	**37** (68%)	**45** (78%)



Scheme 8 Synthesis of final compounds.

our case, the double reaction occurred, but products **46**–**53** were isolated as inseparable mixtures with triphenylphosphine oxide. The reaction was not completed in the case of products **38** and **41** (Table 4, entries 1 and 4). Starting materials were recovered and resubmitted to Mitsunobu conditions. Enantiomers **42** and **44** were not soluble in THF, so the reaction was carried out in THF–toluene–DMF (6 : 1 : 1) solutions (Table 4, entries 5 and 7). The mixtures of products and Ph_3PO were directly submitted to acidic cleavage of the protecting groups. All protecting groups, including benzyl ether, could be cleaved in acidic conditions using TFA–CH_2Cl_2 (3: 1), with a large excess of anisole and pentamethylbenzene as scavengers of benzyl cations.[34] Products **54**–**61** were purified by semi-preparative HPLC and were isolated in 9–40% yields from diols **38**–**45**.

These compounds were tested as CXCR4 receptor antagonists using inhibition of [^{125}I]-SDF-1 binding to CXCR4 transfectants. Compounds **54** and **55** retained activity against CXCR4 receptor (IC_{50} 3.4 and 3.2 μM), despite significant loss of activity when compared to the native peptide (FC131, IC_{50} 0.004 μM).[44] As observed in cyclic pentapeptides, diastereomers **56** and **57**, containing a D configuration for the second Arg, showed no activity. This confirms the importance of this chiral center for antagonist activity. The four compounds containing the less flexible phenol side chain, **58**–**61**, lost all antagonist activity.

Conclusion

We report here the divergent synthesis of tetra-substituted 11-membered ring compounds **54**–**61** from L- and D-glutamic acids. Two of these compounds, **54** and **55**, showed antagonist activity against CXCR4 receptors, and could be potential scaffolds for the development of novel low-molecular-weight CXCR4 antagonists.

Experimental

Methyl (*S*)-2-(*N*-Boc-amino)-5-(triisopropylsilyloxy)pentanoate (L-6) [Boc-Hnv(TIPS)-OMe]

Methyl (*S*)-2-(*N*-Boc-amino)-5-hydroxypentanoate (18.4 g, 74.5 mmol) was dissolved in DMF (100 ml) and was treated with triethylamine (20.8 ml, 2 eq.) and TIPSCl (19.1 ml, 1.2 eq.) and stirred for 5 h at rt. The reaction was partitioned between ether and HCl 0.5 M. The organic layer was washed twice with HCl (0.5 M). The combined aqueous layers were extracted with ether. The combined ether fractions were washed with water and brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using 90 : 10 hexane–ethyl acetate to afford an inseparable mixture (3 : 1) of L-**6** and TIPSOH (29.9 g, 87%). ^{1}H NMR ($CDCl_3$) δ 5.20 (d, 1H, $J = 7.8$ Hz), 4.30 (q, 1H, $J = 7.5$ Hz), 3.73 (s, 3H), 3.70 (t, 2H, $J = 6.3$ Hz), 1.90 (m, 1H), 1.75 (m, 1H), 1.59 (m, 2H), 1.44 (s, 9H), 1.06 (m, 27 H (21H + TIPSOH)). ^{13}C ($CDCl_3$) δ 174.3, 156.2, 80.1, 62.8, 53.6, 52.4, 29.2, 28.8, 28.4, 18.0, 17.8, 12.3, 12.0. FTIR (cm^{-1}) 2941, 2865, 1717, 1503, 1462, 1365, 1166, 1104, 1012, 881. MS (FAB^+) : $m/z = 404$ ($M + H^+$).

(*S*)-2-(*N*-Boc-amino)-5-(triisopropylsilyloxy)pentan-1-ol (L-7). The 3 : 1 mixture of L-**6** and TIPSOH (10 g, 24.8 mmol of **6**) was dissolved in THF (250 ml) and cooled down to −5 °C. DIBAl-H 1M in toluene (87 ml, 4 eq.) was added dropwise over 20 min. The reaction was stirred for an hour at 0 °C. The reaction was quenched by AcOH (5 ml), diluted with HCl 0.5 M (200 ml) and extracted 3 times with EtOAc (100 ml). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (80 : 20 to 50 : 50) to furnish L-**7** (6.46 g, 79%). ^{1}H NMR ($CDCl_3$) δ 4.89

Table 4 Introduction of guanidyl groups and final deprotection

	Diol	*n*	Final product	Yield (2 steps)	*m/z* (calcd), (MH^+)	*m/z* (MH^+)
1	(2*S*,5*S*)-**38**	1	**54**	9%	684.3986	684.3984
2	(2*R*,5*S*)-**39**	1	**55**	19%	”	684.3992
3	(2*R*,5*R*)-**40**	1	**56**	26%	”	684.3993
4	(2*S*,5*R*)-**41**	1	**57**	10%	”	684.3973
5[a]	(2*S*,5*S*)-**42**	0	**58**	40%	670.3829	670.3822
6	(2*R*,5*S*)-**43**	0	**59**	19%	”	670.3821
7[a]	(2*R*,5*R*)-**44**	0	**60**	27%	”	670.3820
8	(2*S*,5*R*)-**45**	0	**61**	15%	”	670.3835

[a] Mitsunobu reaction was done in THF–toluene–DMF (6 : 1 : 1).

(br s, 1H), 3.70–3.56 (m, 5H), 2.71 (br s, 1H), 1.62 (m, 3H), 1.44 (m, 10H), 1.06 (m, 21H). ^{13}C ($CDCl_3$) δ 157.6, 80.0, 66.7, 63.2, 53.1, 29.3, 28.5, 27.8, 18.1, 12.0. FTIR (cm^{-1}) 3339, 2942, 2866, 1687, 1506, 1461, 1390, 1365, 1246, 1170, 1102, 881. $[a]_D^{24}$ −7.9 (c = 5.0, $CHCl_3$), MS (FAB^+) m/z 376 (M + H^+); HRMS calcd for $C_{19}H_{42}NO_4Si^+$ (MH^+) 376.2883, found 376.2887.

***N*-Allyl(*o*-nitrobenzene)sulfonamide.** Nosyl chloride (5.54 g, 25 mmol) was dissolved in DCM (25 ml) and was cooled down to 0 °C. Allylamine (4.7 ml, 62.5 mmol) was added dropwise. The reaction was stirred at rt for 2 h. The solution was washed twice with 10% citric acid solution, with brine, dried over $MgSO_4$ and concentrated to give pure *N*-allyl(*o*-nitrobenzene)sulfonamide (4.42 g, 73%). ^{1}H NMR ($CDCl_3$) δ 8.13 (m, 1H), 7.88 (dd, 1H, J = 3.4 Hz, J = 5.6 Hz), 7.75 (m, 2H), 5.74 (ddt, 1H, J_{d1} = 17.1 Hz, J_{d2} = 10.5 Hz, J_t = 5.6 Hz), 5.41 (br s, 1H), 5.21 (d, 1H, J = 17.1 Hz), 5.11 (d, 1H, J = 10.2 Hz), 3.78 (t, 2H, J = 6.0 Hz).

(*S*)-N^1-Allyl-N^2-Boc-N^1-(*o*-nitrobenzenesulfonyl)-5-(triisopropylsilyloxy)pentane-1,2-diamine (L-8). To a stirred mixture of alcohol L-**7** (6.40 g, 17 mmol), triphenylphosphine (5.36 g, 1.2 eq.) and *N*-allyl(*o*-nitrobenzene)sulfonamide (8.26 g, 2 eq.) in toluene–THF (7 : 1, 40 ml) at 0 °C was added dropwise DIAD solution 1.9 M in toluene (11.7 ml, 1.3 eq.). The reaction was warmed up to rt and stirred for 3 h. The reaction was concentrated and purified by flash chromatography using hexane–EtOAc (90 : 10 to 70 : 30) to furnish product L-**8** (6.25 g, 61%). ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H, J = 6.8 Hz), 7.65 (m, 3H), 5.61 (m, 1H), 5.25 (d, 1H, J = 17.0 Hz), 5.16 (d, 1H, J = 10.2 Hz), 4.62 (d, 1H, J = 9.0 Hz), 4.09 (m, 1H), 3.92 (dd, 1H, J = 7.5 Hz, J = 16.3 Hz), 3.83 (m, 1H), 3.68 (m, 2H), 3.45 (dd, 1H, J = 10.2 Hz, J = 14.4 Hz), 3.21 (dd, 1H, J = 4.9 Hz, 14.6 Hz), 1.61 (m, 3H), 1.41 (m, 10H), 1.05 (m, 21H). ^{13}C ($CDCl_3$) δ 155.8, 147.9, 134.0, 133.4, 132.0, 131.7, 130.9, 124.1, 119.9, 79.2, 62.8, 50.4, 49.6, 47.6, 29.3, 29.1, 28.3, 18.0, 12.0. FTIR (cm^{-1}) 2942, 2866, 1707, 1545, 1509, 1454, 1365, 1244, 1163. MS (FAB^+) m/z 600 (M + H^+); HRMS calcd for $C_{28}H_{50}N_3O_7SSi^+$ (MH^+) 600.3133, found 600.3146. $[a]_D^{21}$ −22.3° (c = 1.04).

Methyl (*S*)-2-(*N*-allylamino)-5-(triisopropylsilyloxy)pentanoate (L-10) [allyl-Hnv(TIPS)-OMe]. Method 1: L-**6** (500 mg, 1.24 mmol) was dissolved in DCM (9 ml) and cooled down to 0 °C. TFA (8 ml) was added dropwise and the reaction was stirred for an hour at 0 °C. The reaction was diluted with toluene and was concentrated. The oil was diluted again in toluene and concentrated to give the TFA salt, which was used without further purification. Dried, powdered 4 Å molecular sieves (2.5 g) were suspended in DMF (13 ml). LiOH (100 mg, 2.13 eq.) was added and the mixture was it stirred for 20 min. Amine was added and the reaction was stirred for 45 additional min. Allyl bromide (100 µl, 1.0 eq.) was finally added and the reaction was stirred overnight. The reaction was filtered, diluted in EtOAc and washed 3 times with water. The combined aqueous layers were extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using DCM–EtOAc (90 : 10) to furnish L-**10** (246 mg, 64%).

Method 2: the 3 : 1 mixture of L-**6** L-**6** and TIPSOH (5.5 g, 13.6 mmol of L-**6**) was dissolved in DCM (70 ml) and cooled down to 0 °C. TFA (60 ml) was added dropwise and the reaction was stirred for an hour at 0 °C. The reaction was diluted with toluene (60 ml) and DCM was removed under reduced pressure. To the solution was carefully added saturated $NaHCO_3$ solution. The aqueous layer was extracted twice with ethyl acetate. The combined organic fractions were washed with brine, dried over $MgSO_4$ and concentrated. The oil was used without further purification. The free amine was dissolved in DCM (20 ml) and was treated with Et_3N (1 ml, 1.2 eq.) followed by nosyl chloride (2.92 g, 1.1 eq.) in solution in DCM (20 ml). The reaction was stirred overnight and was then washed twice with 10% citric acid solution, with brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (90 : 10 to 60 : 40) to furnish methyl (*S*)-2-[*N*-(*o*-nitrobenzenesulfonyl)amino)-5-(triisopropylsilyloxy]pentanoate [Ns-Hnv(TIPS)-OMe] (4.95 g, 85%). ^{1}H NMR ($CDCl_3$) δ 8.07 (m, 1H), 7.92 (m, 1H), 7.73 (m, 2H), 6.10 (d, 1H, J = 9.0 Hz), 4.22 (ddd, 1H, J = 5.4 Hz, J = 8.0 Hz, J = 13.2 Hz), 3.70 (t, 2H, J = 6.1 Hz), 3.47 (s, 3H), 1.99 (m, 1H), 1.82 (m, 1H), 1.63 (m, 2H), 1.05 (m, 21H). ^{13}C ($CDCl_3$) δ 171.6, 136.5, 133.6, 132.9, 130.5, 125.6, 125.3, 62.1, 56.6, 52.3, 29.9, 28.4, 18.0, 12.0. FTIR (cm^{-1}) 2943, 2866, 1742, 1542, 1441, 1358, 1170, 1104.MS (FAB^+) m/z 489 (M + H^+); HRMS calcd for $C_{21}H_{37}N_2O_7SSi^+$ (MH^+) 489.2085, found 489.2096. Nosyl amide (4.90 g, 10 mmol) was dissolved in DMF (40 ml). Allyl bromide (1.3 ml, 1.5 eq.) and K_2CO_3 (1.80 g, 1.3 eq.) were successively added and the reaction was stirred for 3 h at rt. The reaction was partitioned between ether and water. The aqueous phase was extracted twice with ether. The combined organic phases were successively washed with water and brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (90 : 10 to 80 : 20) to furnish methyl (2*S*)-2-[*N*-(*o*-nitrobenzenesulfonyl)-*N*-allylamino]-5-(triisopropylsilyloxy)pentanoate (4.34 g, 82%). ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 1H), 7.67 (m, 2H), 7.59 (m, 1H), 5.93 (ddt, 1H, J_t = 6.8 Hz, J_{d1} = 10.2 Hz, J_{d2} = 17.1 Hz), 5.21 (dd, 1H, J = 1.2 Hz, J = 17.1 Hz), 5.10 (d, 1H, J = 10.2 Hz), 4.66 (dd, 1H, J = 5.4 Hz, J = 10.0 Hz), 4.10 (dd, 1H, J = 5.9 Hz, J = 16.3 Hz), 3.92 (dd, 1H, J = 7.2 Hz, J = 16.3 Hz), 3.71 (t, 2H, J = 5.7 Hz), 3.57 (s, 3H), 2.14 (m, 1H), 1.83 (m, 1H), 1.66 (m, 2H), 1.05 (m, 21H). ^{13}C ($CDCl_3$) δ 171.4, 148.4, 134.9, 133.4, 131.4, 131.2, 124.0, 118.0, 62.3, 60.4, 52.1, 48.8, 29.5, 26.5, 18.0, 11.9. FTIR (cm^{-1}) 2943, 2865, 1743, 1544, 1461, 1437, 1354, 1164, 1101. MS (FAB^+) m/z 529 (M + H^+); HRMS calcd for $C_{24}H_{41}N_2O_7SSi^+$ (MH^+) 529.2398, found 529.2396. The allylnosylamide (4.34 g, 8.21 mmol) was dissolved in DMF (95 ml) and was treated with mercaptoacetic acid (2.8 ml, 5 eq.) and $LiOH{\cdot}H_2O$ (3.40 g, 9.85 eq.). The reaction was stirred for an hour at rt. The reaction was diluted in EtOAc and $NaHCO_3$. Aqueous layer was extracted twice with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (80 : 20) to furnish L-**10** (2.55 g, 90%, 63% from L-**6**). ^{1}H NMR ($CDCl_3$) δ 5.85 (ddt, 1H, J_{d1} = 16.8 Hz, J_{d2} = 10.2 Hz, J_t = 6.1 Hz), 5.17 (dt, 1H, J_d = 17.3 Hz, J_t = 1.5 Hz), 5.08 (dt, 1H, J_d = 10.2 Hz, J_t = 1.5 Hz), 3.72 (s, 3H), 3.68 (t, 2H, J = 6.0 Hz), 3.31–3.24 (m, 2H), 3.11 (ddd, 1H, J = 1.2 Hz, J = 6.1 Hz, J = 13.7 Hz), 1.79 (br s, 1H), 1.72 (m, 2H), 1.59 (m, 2H), 1.05 (m, 21H). ^{13}C ($CDCl_3$) δ 175.9, 136.3, 116.2, 62.9, 60.4, 51.6, 50.7, 29.9, 29.0, 18.0, 11.9. FTIR (cm^{-1}) 2943, 2865, 1737, 1462, 1196, 1171, 1103. MS (FAB^+) m/z 344 (M + H^+); HRMS calcd for $C_{18}H_{38}NO_3Si^+$ (MH^+) 344.2615, found 344.2620.

Methyl (*S*)-2-[*N*-allyl-3-(4-benzyloxyphenyl)propamido]-5-(triisopropylsilyloxy)pentanoate (13a). Amine L-**10** (1.97 g, 5.73 mmol), 3-(4-benzyloxyphenyl)propanoic acid **11** (2.94 g, 2 eq.) and HATU (4.36 g, 2 eq.) were dissolved in DMF (55 ml). Et_3N (2.4 ml, 3 eq.) was added dropwise and the reaction was stirred for 3 h at rt. The reaction was partitioned between ether and HCl 0.5M solution. The aqueous phase was extracted twice with ether. The combined organic phases were successively washed with water and brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (90 : 10 to 80 : 20) to furnish **13a** (3.18 g, 95%). ^{1}H NMR ($CDCl_3$) δ 7.43–7.26 (m, 5H), 7.12 (m, 2H), 6.89 (m, 2H), 5.78 (m, 1H), 5.19–5.13 (m, 2H), 5.03 (s, 2H), 4.87 (dd, 1H, J = 6.1 Hz, J = 9.0 Hz), 3.95 (dd, 1H, J = 5.6 Hz, J = 17.6 Hz), 3.81 (dd, 1H, J = 5.4 Hz, J = 17.8 Hz), 3.68 (m, 5H), 2.92 (m, 2H), 2.62 (dd, 1H, J = 6.6 Hz, J = 8.8 Hz), 2.06 (m, 1H), 1.82 (m, 1H), 1.53 (m, 2H), 1.06 (m, 21H). ^{13}C ($CDCl_3$) δ 173.4, 172.1, 157.2, 137.1, 134.1, 133.6, 132.9, 129.3, 128.6, 127.9, 127.4, 117.2, 114.8, 70.0, 62.7, 57.3, 52.0, 48.8, 35.6, 30.4, 29.8, 25.6, 18.0, 11.9. FTIR (cm^{-1}) 2943, 2865, 1738, 1649, 1611, 1510, 1455, 1238, 1175, 1105.

(*S*)-2-[*N*-Allyl-3-(4-benzyloxyphenyl)propanamido]-5-(triisopropylsilyloxy)pentanoic acid (3a). Ester **13a** (3.18 g, 5.46 mmol) was dissolved in THF–H_2O–MeOH (3 : 1 : 1, 55 ml), cooled down to 0 °C and treated with LiOH·H_2O (688 mg, 3 eq.). The reaction was stirred for 3 h at 0 °C and was diluted with EtOAc and 0.5 M HCl solution. The aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The oil was purified by flash chromatography using hexane–EtOAc (90 : 10 to 60 : 40) to furnish **3a** (2.72 g, 88%). ^{1}H NMR ($CDCl_3$) δ 7.43–7.29 (m, 5H), 7.10 (m, 2H), 6.89 (m, 2H), 5.78 (ddt, 1H, J_{d1} = 17.1 Hz, J_{d2} = 10.2 Hz, J_t = 4.9 Hz), 5.18 (m, 2H), 5.02 (s, 2H), 4.64 (dd, 1H, J = 6.3 Hz, J = 8.5 Hz), 3.96 (dd, 1H, J = 5.6 Hz, J = 17.6 Hz), 3.80 (dd, 1H, J = 5.4 Hz, J = 17.6 Hz), 3.69 (m, 2H), 2.92 (m, 2H), 2.63 (dd, 1H, J = 7.3 Hz, J = 9.0 Hz), 2.12 (m, 1H), 1.87 (m, 1H), 1.56 (m, 2H), 1.05 (m, 21H). ^{13}C ($CDCl_3$) δ 175.7, 174.2, 157.2, 137.1, 134.1, 133.6, 132.9, 129.3, 128.6, 127.9, 127.4, 117.2, 114.8, 69.9, 62.6, 59.0, 50.0, 35.7, 30.4, 29.8, 25.5, 18.0, 11.9. FTIR (cm^{-1}) 2942, 2865, 1731, 1649, 1610, 1545, 1510, 1462, 1239, 1176, 1105. MS (FAB$^+$) m/z 568 (M + H$^+$); HRMS calcd for $C_{33}H_{50}NO_5Si^+$ (MH$^+$) 568.3453, found 568.3454.

(2*R*,*S*)-2-[*N*-Allyl-3-(4-benzyloxyphenyl)propanamido]-*N*-[(2*S*)-1-(*N*-allyl-*o*-nitrophenylsulfonamido)-5-triisopropylsilyloxypentan-2-yl]-5-triisopropylsilyloxypentanamide [(2*R*,*S*,2′*S*)-(14/15)]. L-**8** (3.00 g, 5 mmol) was dissolved in DCM (30 ml) and cooled down to 0 °C. TFA (20 ml) was then added dropwise and the reaction was stirred for an hour at 0 °C. Toluene (30 ml) was added and DCM and TFA were removed under reduced pressure. Toluene solution was diluted with EtOAc and saturated $NaHCO_3$ solution was carefully added. Aqueous phase was extracted twice with EtOAc. Combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated as an oil. L-**4** (2.40 g, 96%) was used without further purification. Acid **3a** (2.00 g, 3.50 mmol), amine L-**4** (2.29 g, 1.3 eq.), HOAt (0.815 g, 1.70 eq.) and HATU (2.68 g, 2.00 eq.) were dissolved in DMF (35 ml) and DIEA (1.23 ml, 2.00 eq.) was added dropwise. The reaction was stirred overnight at room temperature. The reaction was partitioned between ether and HCl 0.5 M. The ether phase was washed again with HCl 0.5 M. The combined aqueous phases were reextracted twice with ether. The combined organic phases were washed with water, brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography using hexane–EtOAc (75 : 25) furnished (**14** + **15**) (3.24 g, 88%) as a 1 : 1 mixture of two diastereomers. MS (FAB$^+$) m/z 1049 (M + H$^+$), 1071 (M + Na$^+$); HRMS calcd for $C_{56}H_{89}N_4O_9SSi_2^+$ (MH$^+$) 1049.5889, found 1049.5879. Analytical HPLC on CHIRACEL OD–H 0.46 × 25 cm in hexane–*i*PrOH (90 : 10 to 5 : 95 in 30 min) give *rt*1 = 9.27 min and *rt*2 = 10.61 min.

(2*R*)- and (2*S*)-2-[*N*-Allyl-(4-benzyloxyphenyl)acetamido]-*N*-[(2*S*)-1-(*N*-allyl-*o*-nitrophenylsulfonamido)-5-triisopropylsilyloxypentan-2-yl]-5-triisopropylsilyloxypentanamide [(2*S*,2′*S*)-18 and (2*R*,2′*S*)-19]. Acid **3b** (1.38 g, 2.49 mmol) and amine L-**4** (1.62 g, 1.3 eq.) were coupled as described for the synthesis of **14/15**. Purification by flash chromatography using hexane–EtOAc (75 : 25) furnished two diastereomers (2*S*,2′*S*)-**18** (1.11 g, 43%) and (2*R*,2′*S*)-**19** (1.23 g, 48%). First to elute (2*S*,2′*S*)-**18**: ^{1}H NMR ($CDCl_3$) δ (signal for major rotamer) 8.01 (m, 1H), 7.66 (m, 3H), 7.46–7.32 (m, 5H), 7.15 (d, 2H, J = 7.5 Hz), 6.93 (d, 2H, J = 7.5 Hz), 6.52 (br d, 1H, J = 9.0 Hz), 5.77 (m, 1H), 5.55 (m, 1H), 5.25–5.08 (m, 4H), 5.04 (s, 2H), 4.57 (br s, 1H), 4.13–3.87 (m, 4H), 3.65 (m, 7H), 3.45 (dd, 1H, J = 10.0 Hz, J = 14.9 Hz), 3.16 (dd, 1H, J = 4.9 Hz, J = 14.9 Hz), 2.01 (m, 1H), 1.89 (m, 1H), 1.48 (m, 6H), 1.05 (m, 42H). MS (FAB$^+$) m/z 1035 (M + H$^+$), 1057 (M + Na$^+$); HRMS calcd for $C_{55}H_{87}N_4O_9Si_2S^+$ (MH$^+$) 1035.5727, found 1035.5726. Second to elute (2*R*,2′*S*)-**19**: ^{1}H NMR ($CDCl_3$) δ 7.96 (m, 1H), 7.68–7.60 (m, 3H), 7.43–7.31 (m, 5H), 7.17 (d, 2H, J = 8.5 Hz), 6.91 (d, 2H, J = 8.5 Hz), 6.28 (d, 1H, J = 9.8 Hz), 5.86 (m, 1H), 5.50 (m, 1H), 5.28 (m, 3H), 5.14 (d, 1H, J = 10.3 Hz), 5.07 (t, 1H, J = 7.8 Hz), 5.03 (s, 2H), 4.12 (m, 2H), 3.99 (dd, 1H, J = 8.0 Hz, J = 16.3 Hz), 3.91 (dd, 1H, J = 4.6 Hz, J = 18.0 Hz), 3.78–3.60 (m, 7H), 3.44 (dd, 1H, J = 10.5 Hz, J = 14.4 Hz), 3.11 (dd, 1H, J = 4.1 Hz, J = 14.4 Hz), 2.01 (m, 1H), 1.74 (m, 1H), 1.50 (m, 6H), 1.04 (m, 42H). MS (FAB$^+$) m/z 1035 (M + H$^+$), 1057 (M + Na$^+$); HRMS calcd for $C_{55}H_{87}N_4O_9SSi_2^+$ (MH$^+$) 1035.5732, found 1035.5743.

(2*S*,5*S*,6*E*)-2,5-Bis[3-(triisopropylsilyloxy)propyl]-1-[3-(4-benzyloxyphenyl)propionyl]-7-(*o*-nitrobenzenesulfonyl)-1,4,7-triazacycloundec-9-en-3-one [(2*S*,5*S*,6*E*)-22] and its (2*R*,5*S*,6*E*)-isomer (23). The diastereomeric mixture **14/15** (3.2 g, 3.05 mmol) was dissolved in DCM (500 ml, 6 mM). The solution was degassed 5 min by bubbling argon and Grubb's I catalyst (250 mg, 0.1 eq.) was added. The reaction was stirred for 12 h under reflux. The catalyst (250 mg, 0.1 eq.) was added again and the reaction was stirred for 12 h under reflux. Volatile compounds were removed and the products were purified by flash chromatography using hexane–EtOAc (70 : 30 to 60 : 40) and furnished two diastereomers, (2*S*,5*S*,6*E*)-**22** (0.406 g, 13%) and (2*R*,5*S*,6*E*)-**23** (0.752 g, 25%). First to elute (2*S*,5*S*,6*E*)-**22** : ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 1H, J = 7.6 Hz), 7.71–7.60 (m, 3H), 7.44–7.29 (m, 5H), 7.13 (d, 2H, J = 8.3 Hz), 6.91 (d, 2H, J = 8.5 Hz), 6.21 (d, 1H, J = 5.9 Hz), 5.57 (d, 1H, J = 16.1 Hz), 5.45 (m, 1H), 5.13 (t, 1H, J = 7.8 Hz), 5.05 (s, 2H), 4.30 (d, 1H, J = 11.5 Hz), 3.92 (d, 1H, J = 9.3 Hz), 3.76 (dd, 1H, J = 5.1 Hz, J = 17.8 Hz), 3.68–3.51 (m, 5H), 3.29 (m, 2H), 3.14 (dd, 1H, J = 9.8 Hz, J = 12.9 Hz), 2.96 (t, 2H, J = 8.5 Hz), 2.65 (t, 2H, J = 8.5 Hz), 2.00 (m, 1H), 1.77 (m, 1H), 1.40 (m, 6H), 1.05 (m, 42H). MS (FAB$^+$) m/z 1021 (M$^+$),

977 ($M^+ - i$Pr). Second to elute (2*R*,5*S*,6*E*)-**23**: ^{1}H NMR ($CDCl_3$) δ 7.95 (dd, 1H, J = 7.5 Hz, J = 1.7 Hz), 7.71–7.56 (m, 3H), 7.41–7.30 (m, 5H), 7.15 (d, 2H, J = 8.5 Hz), 6.91 (d, 2H, J = 8.5 Hz), 5.58 (d, 1H, J = 8.0 Hz), 5.50 (dt, 1H, J_d = 15.8 Hz, J_t = 8.0 Hz), 5.36 (dd, 1H, J = 15.4 Hz, J = 5.1 Hz), 5.20 (t, 1H, J = 7.7 Hz), 5.01 (s, 2H), 3.92 (m, 2H), 3.71–3.63 (m, 7H), 3.54 (d, 1H, J = 4.9 Hz), 3.42 (d, 1H, J = 14.1 Hz), 3.05 (m, 2H), 2.93 (m, 1H), 2.67 (t, 2H, J = 7.5 Hz), 1.85 (m, 1H), 1.72 (m, 2H), 1.48 (m, 5H), 1.05 (m, 42H). MS (FAB^+) m/z 1021 (M^+), 977 ($M^+ - i$Pr).

(2*S*,5*S*,6*E*)-2,5-Bis[3-(triisopropylsilyloxy)propyl]-1-[3-(4-benzyloxyphenyl)propionyl]-7-(2-naphthylacetyl)-1,4,7-triazacycloundec-9-en-3-one [(2*S*,5*S*,6*E*)-30]. (2*S*,5*S*,6*E*)-**22** (612 mg, 0.6 mmol) was dissolved in DMF (6 ml) and was treated with mercaptoacetic acid (0.25 ml, 6 eq.) and $LiOH{\cdot}H_2O$ (280 mg, 11 eq.). The reaction was stirred for an hour at rt. The reaction was diluted in EtOAc and $NaHCO_3$. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The product was used without further purification. It was dissolved in DMF (6 ml) and naphthylacetic acid (223 mg, 2 eq.) and HATU (456 mg, 2 eq.) were added. Et_3N (0.25 ml, 3 eq.) was then added dropwise and the reaction was stirred overnight at rt. Reaction was partitioned between ether and sat. $NaHCO_3$ solution. Aqueous phase was extracted 3× with ether. The combined organic phases were washed with water, brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography using hexane–EtOAc (70 : 30 to 65 : 35) furnished (2*S*,5*S*,6*E*)-**30** (382 mg, 63%): ^{1}H NMR ($CDCl_3$) δ for major rotamer 7.79 (m, 3H), 7.64 (s, 1H), 7.46–7.29 (m, 8H), 7.03 (d, 2H, J = 8.5 Hz), 6.89 (d, 2H, J = 8.7 Hz), 5.93 (d, 1H, J = 6.3 Hz), 5.43 (br d, 1H, J = 19.0 Hz), 5.03 (m, 3H), 4.31 (d, 1H, J = 13.7 Hz), 3.92–3.65 (m, 9H), 3.43–3.32 (m, 3H), 3.23 (dd, 1H, J = 10.7 Hz, J = 14.9 Hz), 2.79 (m, 2H), 2.48 (m, 1H), 2.28 (m, 1H), 1.98 (m, 1H), 1.73 (m, 1H), 1.54 (m, 3H), 1.36 (m, 3H), 1.03 (m, 42H). MS (FAB^+) m/z 1004 (M + H^+), 960 ($M^+ - i$-Pr); HRMS calcd for $C_{60}H_{90}N_3O_6Si_2^+$ (MH^+) 1004.6368, found 1004.6376.

(2*S*,5*S*,6*E*)-2,5-Bis(3-hydroxypropyl)-1-[3-(4-benzyloxyphenyl)-propionyl]-7-(2-naphthylacetyl)-1,4,7-triazacycloundec-9-en-3-one [(2*S*,5*S*,6*E*)-38]. (2*S*,5*S*,6*E*)-**30** (383 mg, 0.38 mmol) was dissolved in THF (3 ml) and was cooled down to −20 °C. TBAF solution (1M, 2 ml, 4 eq.) was added and the reaction was stirred for 48 h. TBAF solution (1M, 1 ml, 2 eq.) was added again and the reaction was stirred for 48 additional hours. The reaction was diluted with EtOAc and was quenched with water. The organic phase was washed twice with water. The combined aqueous phases were extracted 3× with EtOAc–hexane (9 : 1). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography using hexane–EtOAc (50 : 50), hexane–EtOAc–MeOH (48 : 48 : 4) and EtOAc–MeOH (92 : 8) furnished (2*S*,5*S*,6*E*)-**38** (206 mg, 78%): ^{1}H NMR ($CDCl_3$, 40 °C) δ 7.78 (m, 3H), 7.59 (s, 1H), 7.45–7.29 (m, 8H), 7.00 (d, 2H, J = 8.8 Hz), 6.87 (d, 2H, J = 8.5 Hz), 5.47 (m, 1H), 5.07 (m, 1H), 5.01 (s, 2H), 4.26 (d, 1H, J = 13.7 Hz), 3.83 (s, 2H), 3.77 (m, 2H), 3.61–3.50 (m, 5H), 3.31 (m, 3H), 3.20 (dd, 1H, J = 10.7 Hz, J = 14.1 Hz), 2.75 (m, 2H), 2.45 (m, 1H), 2.23 (m, 1H), 1.87 (m, 1H), 1.69–1.40 (m, 7H). MS (FAB^+) m/z 692 (M + H^+); HRMS calcd for $C_{42}H_{50}N_3O_6^+$ (MH^+) 692.3700, found 692.3712.

(2*S*,5*S*,6*E*)-2,5-Bis[(3-guanidino)propyl]-1-[3-(4-hydroxyphenyl)-propionyl]-7-(2-naphthylacetyl)-1,4,7-triazacycloundec-9-en-3-one [(2*S*,5*S*,6*E*)-54]. (2*S*,5*S*,6*E*)-**38** (150 mg, 0.22 mmol), PPh_3 (171 mg, 3 eq.) and *N*,*N*′-bis-Boc-guanidine (337 mg, 6 eq.) were added in a round-bottom flask and submitted to a vacuum/argon (3×). The products were dissolved in THF (3 ml) and the reaction was cooled down to 0 °C. DIAD solution (1.9M, 450 µl, 3.9 eq.) was added dropwise and the reaction was stirred for 24 h at rt. The reaction was partitioned between water and EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography using hexane–EtOAc (70 : 30 to 30 : 70) furnished **46** (approx. 25 mg by NMR) as an inseparable mixture with Ph_3PO and mono-guanidylated product (166 mg). The mono-guanidylated product was resubmitted to Mitsunobu conditions and furnished **22** (approx. 47 mg by NMR) as an inseparable mixture with Ph_3PO. Mixtures of product **46** and Ph_3PO were dissolved in DCM (1.5 ml), treated with anisole (23 eq. based on approx. calculated mass) and pentamethylbenzene (34 eq.). TFA (4.5 ml) was added dropwise and the reaction was stirred for 8 h. The solvent was evaporated and oil was partitioned between water and ether. The water phase was lyophilized to give a yellowish foam which was purified by RP-HPLC in water–acetonitrile (90 : 10 to 40 : 60 in 33 min) to give (2*S*,5*S*,6*E*)-**54** (13 mg, 9%). MS (FAB^+) m/z 684 (M + H^+); HRMS calcd for $C_{37}H_{50}N_9O_4^+$ (MH^+) 684.3986, found 684.3984. $[\alpha]_D^{23}$ −51 (c = 0.1, AcOH).

Biological testing. Stable CHO cell transfectants expressing the CXCR4 variant were prepared as described previously.[35] CHO transfectants were harvested by treatment with citrate saline, and then washed in cold binding buffer (PBS containing 2 mg ml^{-1} BSA). For ligand binding, the cells were resuspended in binding buffer at 1 × 10^7 cells ml^{-1}, and 100 µL aliquots were incubated with 0.1 nM of [^{125}I]-SDF-1 (Perkin-Elmer Life Sciences) for 1 h on ice under constant agitation. Free and bound radioactivities were separated by centrifugation of the cells through an oil cushion, and bound radioactivity was measured with a gamma-counter (Cobra, Packard, Downers Grove, IL). The inhibitory activity of test compounds was determined based on the inhibition of [^{125}I]-SDF-1 binding to CXCR4 transfectants (IC50).

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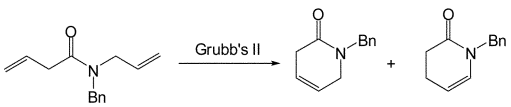

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