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Concise site-specific synthesis of DTPA–peptide conjugates: Application to imaging probes for the chemokine receptor CXCR4

Ryo Masuda, Shinya Oishi *, Hiroaki Ohno, Hiroyuki Kimura, Hideo Saji, Nobutaka Fujii *

Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

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ABSTRACT

Diethylenetriaminepentaacetic acid (DTPA) is a useful chelating agent for radionuclides such as ^{68}Ga, ^{99m}Tc and ^{111}In, which are applicable to nuclear medicine imaging. In this study, we established a facile synthetic protocol for the production of mono-DTPA-conjugated peptide probes. A novel monoreactive DTPA precursor reagent was synthesized in two steps using the chemistry of the *o*-nitrobenzenesulfonyl (Ns) protecting group, and under mild conditions this DTPA precursor was incorporated onto an N^{ε}-bromoacetylated Lys of a protected peptide resin. The site-specific DTPA conjugation was facilitated by using a highly acid-labile 4-methyltrityl (Mtt) protecting group for the target site of the bioactive peptide during the solid-phase synthesis. A combination of both techniques yielded peptides with disulfide bonds, such as octreotide and polyphemusin II-derived CXCR4 antagonists. DTPA–peptide conjugates were purified in a single step following cleavage from the resin and disulfide bond formation. This site-specific on-resin construction strategy was used for the design and synthesis of a novel In-DTPA-labeled CXCR4 antagonist, which exhibited highly potent inhibitory activity against SDF-1–CXCR4 binding.

1. Introduction

Recent progress in molecular imaging methodologies such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and optical imaging technologies has significantly improved the early detection and diagnosis of malignant tumors. To visualize the specific molecular events involved in the physiological and/or pathological processes, a number of peptide-based imaging probes have been developed for overexpressed receptors of peptide hormones and extracellular matrix proteins.[1] These probes are usually designed by a combination of three components: a target-specific vector peptide, an imaging part such as a radionuclide or fluorophore, and a linker to covalently or noncovalently conjugate the peptide with the imaging moiety. The addition of a functional moiety onto small-sized bioactive peptides may be highly susceptible to interaction with receptors or counterpart molecules. Consequently, there have been many reagents of choice for appropriate protein/peptide modifications. In addition, to determine the best labeling position from structure-function relationship studies, versatile synthetic approaches toward various types of labeled peptide are desired.

Polyamino polycarboxylate ligands efficiently coordinate metal radioisotopes to aid the radiolabeling of bioactive peptides. Among the chelating ligands, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) **1a** has been most widely utilized, since a variety of metal radioisotopes for both diagnostic and therapeutic purposes form complexes with high affinity and kinetic stability (Fig. 1).[2] DOTA-modification of bioactive peptides is facilitated by commercially available reagents such as DOTA-NHS **1b** and DOTA-maleimide **1c** to provide the expected peptides in a single step.[3,4] Alternatively, tris(*tert*-butyl)-DOTA **2a** with a free carboxyl group is employed for the modification of an amino group of protected peptides bound to solid-supports.[5] Lysine or phenylalanine derivatives **2b,c** possessing a *tert*-butyl-protected DOTA moiety are also useful components for the peptide sequence assembly.[6] *tert*-Butyl protecting groups in these reagents are easily removed during the final side-chain deprotection process of peptide synthesis.

In contrast to these DOTA derivatives, there has been limited work exploring the application of the diethylenetriaminepentaacetic acid (DTPA) chelating group **3a**, although DTPA represents a promising alternative, especially for ^{68}Ga, ^{99m}Tc and ^{111}In (Fig. 1). The recent success of DTPA-based probes is exemplified by a glucagon-like peptide-1 (GLP-1) receptor ligand, [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-exendin-4, for insulinoma diagnosis.[7] The DTPA group also works as a more favorable functional group than DOTA to facilitate the biological or biodistribution properties of several probes.[8] For the preparation of DTPA-conjugated imaging probes, several conjugation reagents have been developed. The most familiar cyclic diethylenetriaminepentaacetic dianhydride **4**

* Corresponding authors. Tel.: +81 75 753 4551; fax: +81 75 753 4570.
E-mail addresses: soishi@pharm.kyoto-u.ac.jp (S. Oishi), nfujii@pharm.kyoto-u.ac.jp (N. Fujii).

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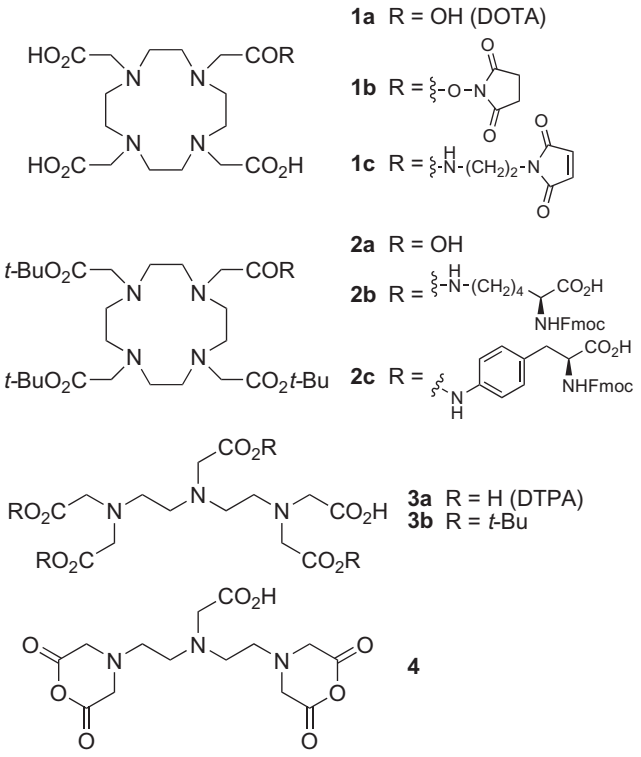


Figure 1. Structures of radionuclide chelating agents and the precursors.

is a bifunctional chelating agent, which can conjugate with peptide hormones and antibodies.[9] Using this reagent, concomitant formations of a bis-conjugated product[10] and intra- and intermolecular cross-linked products[11] were unavoidable. Monoreactive DTPA derivatives have also been developed for the preparation of DTPA–peptide conjugates without the unfavorable by-product formations.[12,13] For example, we reported the synthesis and application of 3,6,9,9-tetrakis[(*tert*-butoxycarbonyl)methyl]-3,6,9-triazanonanoic acid **3b** (mDTPA),[14] in which the four carboxylates were protected with *tert*-butyl ester. However, a longer process from the commercially available reagents is required for the synthesis of these DTPA-conjugation reagents (Scheme 1A).

Accordingly, to establish a facile and efficient synthetic method for DTPA–peptide conjugates, we have investigated the site-specific and on-resin construction of a DTPA moiety. Herein, we describe the short-step synthesis of a DTPA precursor using the *o*-nitrobenzenesulfonyl (Ns) protecting group and the solid-phase synthesis of DTPA–peptide conjugates. The design and synthesis of DTPA–peptide conjugates that potentially target the somatostatin receptor and chemokine receptor CXCR4 are also presented.[15]

2. Results and discussion

2.1. Synthesis of a DTPA-conjugation reagent and the application to octreotide derivatives

The synthetic scheme for the production of mDTPA reagent **10**, as described in our previous study, is presented in Scheme 1A. We hypothesized that two remedies could significantly improve the overall synthetic process of DTPA–peptide conjugates. First, the use of an Ns group in place of the trifluoroacetyl group was expected to serve as a temporary protecting group and an auxiliary group for global modification with four *tert*-butoxycarbonylmethyl groups. This potentially improves the stepwise synthesis of the intermediate **7** in the solution-phase. In addition, a secondary amine **8** as a nucleophilic precursor for the bromoacetyl group on peptide resin **11** can directly produce the overall DTPA framework of **12** on the solid support without the additional three-step modification process of **8** in solution (Scheme 1B).[16]

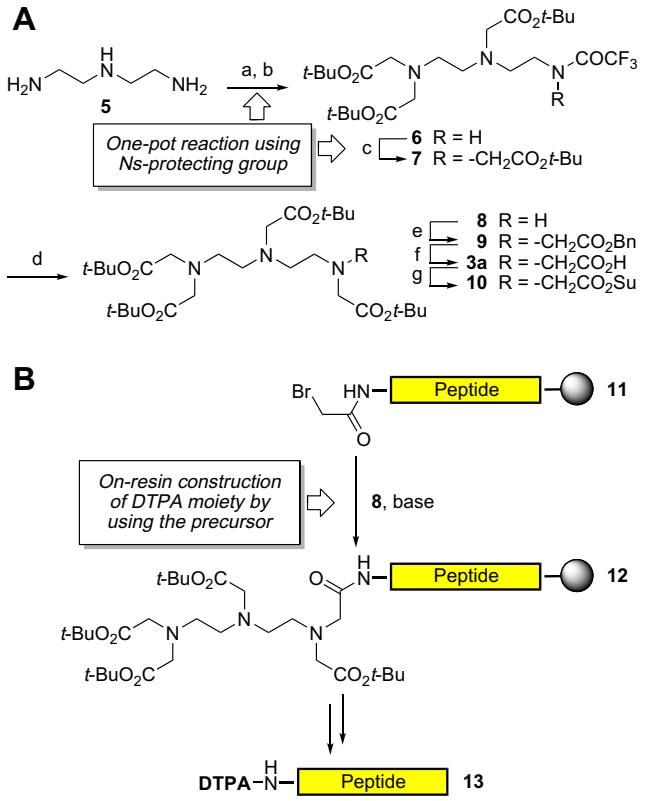
Synthesis of DTPA precursor **8** began with mono-Ns protection of the commercially available diethylenetriamine **5** (Scheme 2). The Ns-protected intermediate was successively treated with excess equivalent of *t*-butyl bromoacetate in a one-pot process. Although the solvent EtOH has been reported to be effective in predominantly giving the mono-Ns product,[17] concomitant production of bis-Ns product **14b** was not suppressed as in DMF. The treatment of excess diethylenetriamine **5** with NsCl in EtOH provided mono-Ns product **14a** in 65% yield (calculated based on NsCl), which can be readily purified by chromatography. Compound **14a** was then subjected to deprotection with mercaptoacetic acid and LiOH to provide the expected precursor **8** in 77% yield.

Using the resulting reagent **8**, DTPA-conjugation of [D-Phe^1]octreotide was investigated as a model study (Scheme 3), which is employed as a radionuclide imaging probe for the somatostatin receptor.[14,18,19] After peptide-chain elongation by Fmoc-based solid-phase peptide synthesis, the N-terminus of **16** was modified with bromoacetic acid and 1,3-diisopropylcarbodiimide (DIC). Subsequently, the bromide **17** was treated with the reagent **8** in the presence of $(i\text{-Pr})_2$NEt to provide the fully protected peptide resin **18a**. Cleavage from the resin **18a** and disulfide formation under air-oxidation conditions provided [DTPA-D-Phe^1]octreotide **19a** with high purity. The bromoacetylated peptide **17** was also modified with commercially available DOTA precursor reagent **20**, using the identical procedure to provide [DOTA-D-Phe^1]octreotide **19b**.[20] These suggest that this on-resin modification procedure is widely applicable to any chelating reagents with nucleophilic functional groups such as DTPA and DOTA precursors.

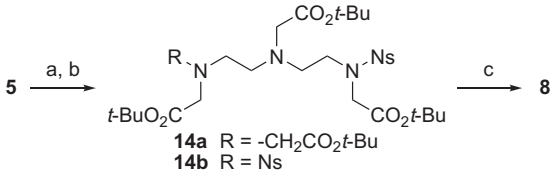
2.2. Site-specific DTPA-conjugation of bioactive peptides: synthesis of CXCR4 receptor probes

It has been reported that a high level of CXCR4 expression in tumors is associated with malignant and metastatic properties.[21] Intrinsic SDF-1 release from the potential distal metastatic sites mediates organ-specific metastasis of CXCR4-expressing cells from the primary lesions. Since CXCR4-expressing cancer stem cells are related to the metastatic spread in orthotopic primary tumors,[22] it is of considerable importance to develop potent CXCR4-imaging probes to detect potential cancer stem cells within malignant tumors, as exemplified by the diagnosis of bladder cancer by a fluorescent CXCR4 probe.[23,24]

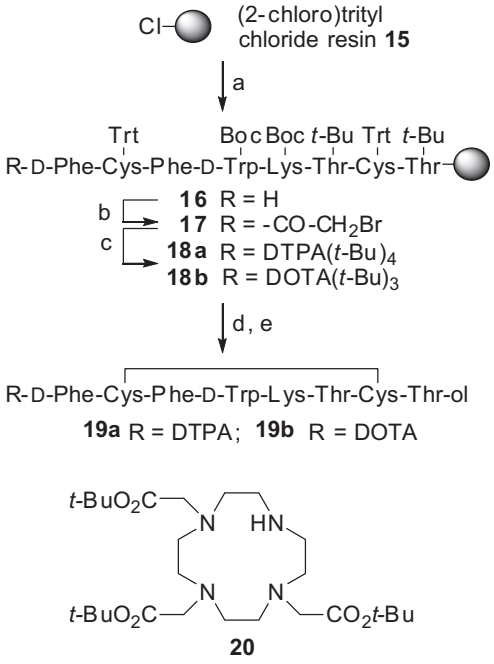
Previously, we reported a DTPA-conjugated CXCR4 antagonist, DTPA-Ac-TZ14011 **26a**,[25] which was designed from a horseshoe crab-derived anti-HIV peptide T140. This peptide has β-sheet-like structures maintained by a disulfide bond, around which the pharmacophore residues for bioactivity are located.[26] For the site-specific conjugation at D-Lys^8 in the type II' β-turn region of T140 with a single DTPA group in the solution-phase, a secondary lysine (Lys^7) was substituted with arginine, which cannot be acylated by standard reagents.[25] Although a DTPA group was successfully ligated with maintenance of highly potent CXCR4 antagonistic activity in this case,[25] the accompanying substitutions needed for specific modification of other peptides may possibly lead to a decrease in the bioactivity. Therefore, we planned the facile site-specific DTPA conjugation on a solid-support for production of CXCR4 imaging probes without substitution of the secondary Lys^7 residue. To distinguish D-Lys^8 to be labeled in peptides **26**, the highly acid-labile 4-methyltrityl (Mtt) group was exploited for temporary protection of the ε-amino group during solid-phase peptide synthesis.[27] For the other Lys residues such as Lys^7 of



Scheme 1. (A) Synthetic scheme for the DTPA-conjugation reagent **10** prepared in our previous study; (B) synthetic plan for the DTPA-conjugated peptides in this study. Reagents: (a) CF_3CO_2Et; (b) $BrCH_2CO_2t$-Bu, $(i\text{-}Pr)_2NEt$; (c) $BrCH_2CO_2t$-Bu, NaH; (d) NH_2NH_2, *t*-BuOH; (e) $BrCH_2CO_2Bn$, $(i\text{-}Pr)_2NEt$; (f) H_2, Pd/C; (g) DCC, HOSu.

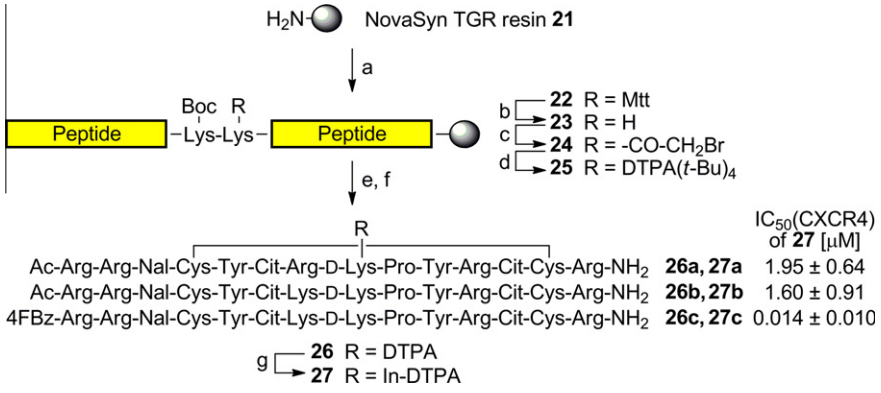


Scheme 2. Synthesis of DTPA precursor **8** via a global N-alkylation process using a Ns-protecting group. Reagents: (a) NsCl; (b) $BrCH_2CO_2t$-Bu, K_2CO_3; (c) $HSCH_2CO_2H$, LiOH.



Scheme 3. Synthesis of DTPA- and DOTA-conjugated D-Phe-octreotides. Reagents: (a) Fmoc-based peptide synthesis; (b) $BrCH_2CO_2H$, DIC; (c) **8** for **18a**, or **20** for **18b**, $(i\text{-}Pr)_2NEt$ (d) TFA/H_2O/1,2-ethanedithiol (EDT) (95:2.5:2.5) for **19a**, 1 M TMSBr, thioanisole/TFA, 1,2-ethanedithiol, *m*-cresol for **19b**; (e) NH_4OH (air oxidation).

26b,c, a Boc group was employed. This group can be cleaved by the standard TFA-based treatment in Fmoc chemistry (Scheme 4). After the construction of the protected peptide resin, the orthogonal Mtt group at the labeling position was cleaved off using 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP). The resulting ε-amino group was successively modified with bromoacetic acid followed by the reagent **8** to provide the fully protected DTPA–peptide resin **25**. Final deprotection, air-oxidation and HPLC purification afforded the expected DTPA-conjugated CXCR4 antagonists **26a,b**. This concise protocol facilitates the selection of chelating structure and position(s) on the peptide chain, and aids structure–activity relationship studies aimed at exploring the more potent peptide probes. For example, a 4-fluorobenzoyl modification at the N-terminus, which should increase CXCR4 antagonism,[28] was easily appended to the peptide using this protocol to give the modified peptide **26c**. The subsequent treatment with nonradioactive $InCl_3$ in acidic conditions provided the In-DTPA-labeled CXCR4 antagonists **27a–c**.



Scheme 4. Site-specific In-DTPA labeling of CXCR4 antagonists and biological activity. Reagents: (a) Fmoc-based peptide synthesis; (b) CH_2Cl_2/1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)/2,2,2-trifluoroethanol (TFE)/triethylsilane (TES) (65:20:10:5); (c) $BrCH_2CO_2H$, DIC; (d) **8**, $(i\text{-}Pr)_2NEt$; (e) TFA/H_2O/EDT (95:2.5:2.5); (f) NH_4OH (air oxidation); (g) $InCl_3$. *Abbreviations:* Mtt: 4-methyltrityl; Cit: L-citrulline, Nal: L-3-(2-naphthyl)alanine, 4FBz: 4-fluorobenzoyl.

2.3. Bioactivity of In-DTPA-labeled CXCR4 antagonists

The biological activity of the In-DTPA-labeled peptides **27a–c** was evaluated as the inhibitory potency of [^{125}I]-SDF-1-binding to CXCR4 membrane extracts (Scheme 4). Peptides **27a,b**, with an N-terminal acetyl group, exhibited similar potency towards CXCR4 [IC_{50}(**27a**) = 1.95 ± 0.64 μM, IC_{50}(**27b**) = 1.60 ± 0.91 μM], indicating that the Lys and Arg for the *i*-position of β-turn were both tolerant to the bioactivity. In contrast, peptide **27c** exerted much more potent inhibitory activity for the SDF-1 binding to CXCR4 [IC_{50}(**27c**) = 0.014 ± 0.010 μM]. These results of In-DTPA-labeled peptides **27a–c** coincided with our previous report on the unlabeled peptides.[28] The novel potent In-DTPA-labeled CXCR4 antagonist **27c** could be a promising imaging probe for CXCR4-expressing malignant cancer cells.[11]

3. Conclusions

In this study, we have established a novel synthetic method for the production of DTPA–peptide conjugates. The process includes facile solid-phase synthesis of a DTPA framework using a novel precursor substrate and site-specific conjugation using a highly acid-labile protecting group. Using a temporary Ns protecting group, the DTPA precursor **8** was obtained through two purification steps from commercially available diethylenetriamine. In addition, the on-resin incorporation of a bromoacetyl group into the specific free amino group followed by the addition of the nucleophilic DTPA precursors provided the expected DTPA–peptide conjugates with high purity. Taking advantage of secondary amine precursors of choice, these processes represent versatile methods to prepare a series of peptide conjugates, including DTPA and DOTA, for optimization of imaging probes. This conjugation method was applied to the preparation of DTPA-conjugates of octreotide and CXCR4 antagonist, which have been reported to effectively detect cancer cells. The peptide **27c** with highly potent inhibitory activity of SDF-1 binding to CXCR4 was obtained without any amino acid substitution to avoid multiple modifications on the amino groups. This peptide represents a promising lead compound as an imaging probe towards CXCR4-positive metastatic tumors.

4. Experimental

4.1. Synthesis

4.1.1. Bis(*tert*-butyl) 3,6-bis[(*tert*-butoxycarbonyl)methyl]-9-(*o*-nitrobenzenesulfonyl)-3,6,9-triazaundecanedioate (14a)

To diethylenetriamine **5** (0.540 mL, 5.00 mmol) in dehydrated EtOH (5 mL), *o*-NsCl (0.367 g, 1.67 mmol) was slowly added below 0 °C. After stirring for 2 h, EtOH was removed in vacuo. To dehydrated DMF (8 mL), K_2CO_3 (4.49 g, 32.5 mmol) and $BrCH_2CO_2t$-Bu (4.06 mL, 27.5 mmol) were added at 0 °C. The mixture was stirred overnight at room temperature, and filtered. The filtrate was concentrated under reduced pressure to give an oily residue, and the residue was dissolved in EtOAc (100 mL). The whole mixture was washed with saturated $NaHCO_3$, and was dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–EtOAc gave compound **14a** as a yellow oil (0.81 g, 65%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08–8.11 (1H, m), 7.64–7.69 (2H, m), 7.56–7.60 (1H, m), 4.24 (2H, s), 3.49 (2H, t, *J* = 6.9 Hz), 3.42 (4H, s), 3.30 (2H, s), 2.88 (2H, t, *J* = 6.6 Hz), 2.78 (2H, t, *J* = 6.9 Hz), 2.77 (2H, t, *J* = 6.9 Hz), 1.45 (27H, s), 1.36 (9H, s); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 170.6 (3C), 168.0, 133.7, 133.2, 131.6 (2C), 130.9, 123.9, 82.0, 81.0, 80.9 (2C), 56.1 (3C), 53.3, 52.8, 52.4, 49.4, 46.7, 28.1 (9C), 27.9 (3C); HRMS (FAB) *m*/*z* calcd for $C_{34}H_{58}N_4O_{12}S$ ([M+H]$^+$): 746.3772, found 746.3779.

4.1.2. Bis(*tert*-butyl) 3,6-bis[(*tert*-butoxycarbonyl)methyl]-3,6,9-triazaundecanedioate (8)

To a solution of compound **14a** (0.216 g, 0.29 mmol) in DMF (0.726 mL), LiOH (0.128 g, 2.90 mmol) and mercaptoacetic acid (0.101 mL, 1.45 mmol) were added below 0 °C. After stirring for 2 h at room temperature, the mixture was concentrated under reduced pressure, and the residue was dissolved in $CHCl_3$. The whole reaction mixture was washed with saturated $NaHCO_3$, and was dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with $CHCl_3$–MeOH gave compound **8** as a yellow oil (0.124 g, 77%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.39 (4H, s), 3.28 (4H, s), 2.72–2.82 (6H, m), 2.63 (2H, t, *J* = 5.4 Hz), 1.39 (9H, s), 1.38 (27H, s); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 170.9, 170.7 (3C), 80.8 (4C), 55.9 (2C), 55.8 (2C), 52.4, 52.3, 51.3, 47.0, 28.2 (3C), 28.1 (9C); HRMS (FAB) *m*/*z* calcd for $C_{28}H_{54}N_3O_8$ ([M+H]$^+$): 560.3911, found 560.3910.

4.1.3. Standard procedure for solid-phase peptide synthesis

Protected peptide-resins were manually constructed by Fmoc-based solid-phase peptide synthesis. *t*-Bu ester for Asp and Glu; 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl (Pbf) for Arg; *t*-Bu for Thr and Tyr; Boc for Lys and Trp; Trt for Cys were employed for side-chain protection. Fmoc-amino acids were coupled using three equivalents of reagents [Fmoc-amino acid, 1,3-diisopropylcarbodiimide (DIC), and HOBt·H_2O] to the free amino group in DMF for 1.5 h. Fmoc deprotection was performed by 20% (v/v) piperidine in DMF (2 × 1 min, 1 × 30 min). The protected peptide resin was treated with a cocktail of deprotection reagents. After removal of the resin by filtration, the filtrate was poured into ice-cold dry Et_2O. The resulting powder was collected by centrifugation and washed with ice-cold dry Et_2O. The crude peptide was dissolved in H_2O, and the pH was adjusted to 8.0 with NH_4OH for disulfide bond formation. After air-oxidation for 1 d, the crude product was purified by preparative HPLC on a Cosmosil 5C18-ARII preparative column (Nacalai Tesque, Kyoto, Japan; 20 × 250 mm, flow rate 10 mL/min) to afford the expected peptides. All peptides were characterized by MALDI-TOF-MS (AXIMA-CFR plus, Shimadzu, Kyoto, Japan) and the purity was calculated as >95% by HPLC on a Cosmosil 5C18-ARII analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1 mL/min) at 220 nm absorbance.

4.1.4. Preparation of DTPA- and DOTA-conjugated octreotides (19a,b)

According to the procedure reported previously,[18] (2-chloro)trityl chloride resin **15** (214 mg, 1.4 mmol/g), Fmoc-Thr(*t*-Bu)-ol (345 mg, 0.9 mmol), and pyridine (0.145 mL, 1.8 mmol) were agitated for 21 h in dry CH_2Cl_2–DMF (1:1, 3.94 mL). The loading was determined by measuring the 290 nm UV absorption of the piperidine-treated sample (0.455 mmol/g). After the construction of the peptide chain (0.017 mmol scale) using a standard procedure, bromoacetic acid (23.6 mg, 0.17 mmol) with DIC (0.026 mL, 0.17 mmol) in CH_2Cl_2 was reacted with resin **16** for 2 h at room temperature. The subsequent treatment of **17** with amines **8** (29.0 mg, 0.51 mmol) and **20** (26.3 mg, 0.51 mmol) with (*i*-Pr)$_2$NEt (0.009 mL, 0.51 mmol) in DMF for 12 h at room temperature provided **18a** and **18b**, respectively. Cleavage and deprotection of **18a** (72.5 mg) and **18b** (73.8 mg) was achieved using a TFA/1,2-ethandithiol (EDT)/H_2O (5 mL; 95:2.5:2.5) cocktail for 2 h at room temperature and by treatment with 1 M TMSBr-thioanisole/TFA in the presence of EDT/*m*-cresol (3.3 mL) for 2 h at 0 °C, respectively. After disulfide formation under air-oxidation conditions, the crude peptides were purified using the standard procedure, to afford the desired peptides **19a** (8.2 mg, 23%) and **19b** (9.5 mg, 26%) as white powders. Compound **19a**: MS (MALDI-TOF) *m*/*z* calcd for $C_{63}H_{89}N_{13}O_{19}S_2$ ([M+H]$^+$): 1395.6, found 1395.3. Compound **19b**:

MS (MALDI-TOF) m/z calcd for $C_{65}H_{93}N_{14}O_{17}S_2$ ($[M+H]^+$): 1405.6, found 1405.8.

4.1.5. Preparation of DTPA-conjugated CXCR4 antagonists (26a–c)

Protected peptide resins were manually constructed according to the standard procedure using NovaSyn TGR-resin **21** (96.2 mg, 0.025 mmol). 4-Methyltrityl (Mtt) group was employed for the protection of the D-Lys ε-amino group. The N-terminal amino group was acylated by treatment with Ac_2O (0.012 mL, 0.125 mmol)/pyridine (0.020 mL, 0.250 mmol) for 1 h at room temperature for peptides **26a**,**b**, and with 4-fluorobenzoic acid (17.5 mg, 0.125 mmol)/DIC (0.019 mL, 0.125 mmol)/HOBt·H_2O (19.2 mg, 0.125 mmol) for 1.5 h at room temperature for peptide **26c**. Subsequently, the resin **22** was treated with CH_2Cl_2/1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP)/trifluoroethanol (TFE)/triethylsilane (TES) [65:20:10:5; 5 mL] for 2 h at room temperature. The DTPA group was incorporated using the identical procedure employed for the synthesis of the octreotide derivative **19a**. Treatment of the resins (**25a**: 178 mg, **25b**: 165 mg, **25c**: 162 mg) with a TFA/1,2-ethandithiol(EDT)/H_2O (95:2.5:2.5; 5 mL) cocktail for 2 h at room temperature followed by air oxidation and purification provided the peptides Compound **26a** (14.6 mg, 15.4%), **26b** (6.67 mg, 8.7%) and **26c** (7.4 mg, 9.5%) as white powders. Compound **26a:** MS (MALDI-TOF) m/z calcd for $C_{106}H_{165}N_{38}O_{28}S_2$ ($[M+H]^+$): 2482.2, found 2482.5. Compound **26b**: MS (MALDI-TOF) m/z calcd for $C_{106}H_{165}N_{36}O_{28}S_2$ ($[M+H]^+$): 2454.2, found 2453.9. Compound **26c**: MS (MALDI-TOF) m/z calcd for $C_{111}H_{166}FN_{36}O_{28}S_2$ ($[M+H]^+$): 2534.2, found 2533.8.

4.1.6. Indium chelating for CXCR4 antagonist probes (27a–c)

To a solution of peptides **26a**–**c** (8 mM in 0.1 N AcOH, **26a**: 45.9 μL, 0.37 μmol; **26b**: 48.4 μL, 0.39 μmol; **26c**: 48.8 μL, 0.39 μmol), $InCl_3$ (1 M in 0.02 N HCl, 50 μL) was added and the solution stirred for a further 30 min at room temperature. HPLC purification using a standard procedure provided the desired peptides **27a** (0.43 mg, 36.7%), **27b** (0.42 mg, 34.3%) and **27c** (0.38 mg, 30.3%) as white powders. Compound **27a**: MS (MALDI-TOF) m/z calcd for $C_{106}H_{165}InN_{38}O_{28}S_2$ ($[M+H]^+$): 2597.1, found 2596.9. Compound **27b**: MS (MALDI-TOF) m/z calcd for $C_{106}H_{165}InN_{36}O_{28}S_2$ ($[M+H]^+$): 2569.1, found 2569.1. Compound **27c**: MS (MALDI-TOF) m/z calcd for $C_{111}H_{166}FInN_{36}O_{28}S_2$ ($[M+H]^+$): 2649.1, found 2649.0.

4.2. Evaluation of [^{125}I]-SDF-1 binding and displacement

For ligand binding, the CXCR4 membrane was incubated with 0.5 nM of [^{125}I]-SDF-1 and increasing concentrations of compounds **27a**–**c** in binding buffer [50 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.1% BSA in H_2O] for 1 h at room temperature. The reaction mixtures were filtered through GF/B filters (Perkin-Elmer, Wellesley, MA) pretreated with 0.1% polyethyleneimine. The filter plate was washed with wash buffer [50 mM HEPES (pH 7.4), 500 mM NaCl and 0.1% BSA in H_2O] and the bound radioactivity was measured by TopCount (Packard, Meriden, CT). Inhibitory activity of test compounds was determined based on the inhibition of [^{125}I]-SDF-1 binding to the CXCR4 receptor (IC_{50}).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.059.

References and notes

1. Lee, S.; Xie, J.; Chen, X. *Chem. Rev.* **2010**, *110*, 3087.
2. De León-Rodríguez, L. M.; Kovacs, Z. *Bioconjugate Chem.* **2008**, *19*, 391.
3. Mier, W.; Hoffend, J.; Krmer, S.; Schuhmacher, J.; Hull, W. E.; Eisenhut, M.; Haberkorn, U. *Bioconjugate Chem.* **2005**, *16*, 237.
4. Lewis, M. R.; Shively, J. E. *Bioconjugate Chem.* **1998**, *9*, 72.
5. Heppeler, A.; Froidevaux, S.; Mäcke, H. R.; Jermann, E.; Powell, P.; Henning, M. *Chem. Eur. J.* **1999**, *5*, 1974.
6. De León-Rodriguez, L. M.; Kovacs, Z.; Dieckmann, G. R.; Sherry, A. D. *Chem. Eur. J.* **2004**, *10*, 1149.
7. Wild, D.; Wicki, A.; Mansi, R.; Béhé, M.; Keil, B.; Bernhardt, P.; Christofori, G.; Ell, P. J.; Mäcke, H. R. *J. Nucl. Med.* **2010**, *51*, 1059, and the references therein.
8. De Jong, M.; Breeman, W. A.; Bakker, W. H.; Kooij, P. P.; Bernard, B. F.; Hofland, L. J.; Visser, T. J.; Srinivasan, A.; Schmidt, M. A.; Erion, J. L.; Bugaj, J. E.; Mäcke, H. R.; Krenning, E. P. *Cancer Res.* **1998**, *58*, 437.
9. Hnatowich, D. J.; Layne, W. W.; Childs, R. L. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 327.
10. Wang, S.; Luo, J.; Lantrip, D. A.; Waters, D. J.; Mathias, C. J.; Green, M. A.; Fuchs, P. L.; Low, P. S. *Bioconjugate Chem.* **1997**, *8*, 673.
11. Reilly, R.; Lee, N.; Houle, S.; Law, J.; Marks, A. *Appl. Radiat. Isot.* **1992**, *43*, 961.
12. Hnatowich, D. J.; Layne, W. W.; Childs, R. L.; Lanteigne, D.; Davis, M. A.; Griffin, T. W.; Doherty, P. W. *Science* **1983**, *220*, 613.
13. Van Hagen, P. M.; Breeman, W. A. P.; Bernard, H. F.; Schaar, M.; Mooij, C. M.; Srinivasan, A.; Schmidt, M. A.; Krenning, E. P.; De Jong, M. *Int. J. Cancer* **2000**, *90*, 186.
14. Arano, Y.; Uezono, T.; Akizawa, H.; Ono, M.; Wakisaka, K.; Nakayama, M.; Sakahara, H.; Konishi, J.; Yokoyama, A. *J. Med. Chem.* **1996**, *39*, 3451.
15. A portion of this study was reported in a preliminary communication: Masuda, R.; Ohno, H.; Oishi, S.; Fujii, N. In *Pepide Science*, Okamoto, Ed.; 2009, p 159.
16. Peterson, J. J.; Pak, R. H.; Meares, C. F. *Bioconjugate Chem.* **1999**, *10*, 316.
17. Hidai, Y.; Kan, T.; Fukuyama, T. *Chem. Pharm. Bull.* **2000**, *48*, 1570.
18. Arano, Y.; Akizawa, H.; Uezono, T.; Akaji, K.; Ono, M.; Funakoshi, S.; Koizumi, M.; Yokoyama, A.; Kiso, Y.; Saji, H. *Bioconjugate Chem.* **1997**, *8*, 442.
19. Lewis, J. S.; Anderson, C. J. *Methods Mol. Biol.* **2007**, *386*, 227.
20. Albert, R.; Smith-Jones, P.; Stolz, B.; Simeon, C.; Knecht, H.; Bruns, C.; Pless, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1207.
21. Müller, A.; Homey, B.; Soto, H.; Ge, N.; Catron, D.; Buchanan, M. E.; McClanahan, T.; Murphy, E.; Yuan, W.; Wagner, S. N.; Barrera, J. L.; Mohar, A.; Verástegui, E.; Zlotnik, A. *Nature* **2001**, *410*, 50.
22. Hermann, P. C.; Huber, S. L.; Heeschen, C. *Cell Cycle* **2008**, *7*, 188.
23. Oishi, S.; Masuda, R.; Evans, B.; Ueda, S.; Goto, Y.; Ohno, H.; Hirasawa, A.; Tsujimoto, G.; Wang, Z.; Peiper, S. C.; Naito, T.; Kodama, E.; Matsuoka, M.; Fujii, N. *ChemBioChem* **2008**, *9*, 1154.
24. Nishizawa, K.; Nishiyama, H.; Oishi, S.; Tanahara, N.; Kotani, H.; Mikami, Y.; Toda, Y.; Evans, B. J.; Peiper, S. C.; Saito, R.; Watanabe, J.; Fujii, N.; Ogawa, O. *Int. J. Cancer* **2010**, *127*, 1180.
25. Hanaoka, H.; Mukai, T.; Tamamura, H.; Mori, T.; Ishino, S.; Ogawa, K.; Iida, Y.; Doi, R.; Fujii, N.; Saji, H. *Nucl. Med. Biol.* **2006**, *33*, 489.
26. Tamamura, H.; Omagari, A.; Oishi, S.; Kanamoto, T.; Yamamoto, N.; Peiper, S. C.; Nakashima, H.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2633.
27. Stephenson, K. A.; Banerjee, S. R.; McFarlane, N.; Boreham, D. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *Can. J. Chem.* **2005**, *83*, 2060.
28. Tamamura, H.; Hiramatsu, K.; Mizumoto, M.; Ueda, S.; Kusano, S.; Terakubo, S.; Akamatsu, M.; Yamamoto, N.; Trent, J. O.; Wang, Z.; Peiper, S. C.; Nakashima, H.; Otaka, A.; Fujii, N. *Org. Biomol. Chem.* **2003**, *1*, 3663.